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# Integrated catalytic processes for the treatment of organic pollutants and inactivation of microorganisms

**Nuno Filipe Figueiredo Moreira**

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**President of the Jury:**

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**Supervisor:** Adrián Manuel Tavares da Silva, Investigador Principal

**Co-supervisor:** Olga Cristina Pastor Nunes, Professora Auxiliar

**Co-supervisor:** Manuel Fernando Ribeiro Pereira, Professor Associado

Department of Chemical Engineering
Faculty of Engineering - University of Porto

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# Abstract

The aim of this work is to study the degradation of organic pollutants and inactivation of microorganisms through advanced oxidation processes, including their integration in a single reactor. The selected model emerging pollutants, amoxicillin and diclofenac, were subjected to single ozonation, photolysis, photolytic ozonation and photocatalytic ozonation. A complete degradation of amoxicillin occurred in less than 10 min for all experiments where ozone was employed, i.e. single ozonation, photolytic ozonation and photocatalytic ozonation. However, this organic pollutant is very stable under direct photolysis. Diclofenac was completely degraded (after 5 min) regardless the AOP tested.

Oxalic and oxamic acids were detected as typical reaction intermediates of amoxicillin and diclofenac degradation. These acids, as well as carbon dioxide, probably contribute to a decrease in pH during the experiments. The total organic carbon measurements confirmed a complete mineralization of amoxicillin and diclofenac (after 30 and 120 min, respectively) when using the photocatalytic ozonation process. Nitrates and ammonium were typical final oxidation products of both pollutants, while sulfates for amoxicillin and chlorides for diclofenac were also detected.

The transformation of amoxicillin and diclofenac into non-toxic compounds after the photocatalytic ozonation treatment was confirmed in growth inhibition assays using two different strains, *Escherichia coli* and *Staphylococcus aureus.* It was found that the antimicrobial activity of amoxicillin was lost after 15 min. On the other hand, no toxic effect was observed for diclofenac neither for degradation products thereof.

Inactivation assays permitted to conclude that the photolytic ozonation process is effective in disinfection of water, allowing the complete elimination of *Escherichia coli*, after 180 min of treatment.

In this context, the photocatalytic ozonation process is an efficient solution for simultaneous degradation of organic pollutants and inactivation of microorganisms, of major relevance for the development of water treatment technologies.

# Resumo

O principal objetivo deste trabalho consistiu em estudar a degradação de contaminantes orgânicos e a inativação de microrganismos através de processos avançados de oxidação, incluindo a integração destes processos num único reator. Os poluentes emergentes estudados, amoxicilina e diclofenac, foram sujeitos a ozonização simples, fotólise, ozonização fotolítica e ozonização fotocatalítica. Relativamente à amoxicilina, verificou-se a completa degradação deste poluente em menos de 10 min para todas as experiências em que foi utilizado ozono. Foi verificada uma degradação pelo processo de fotólise direta inferior a 5%. O diclofenac foi completamente degradado após 5 min, em todas as reações.

O ácido oxálico e oxâmico foram comummente identificados como compostos intermediários e, juntamente com o dióxido de carbono formado, contribuem para uma diminuição no valor do pH durante as experiências. As análises de remoção de carbono orgânico total permitiram confirmar a completa mineralização da amoxicilina e do diclofenac (após 30 e 120 min, respetivamente) no processo de ozonização fotocatalítica. Nitratos e amónia foram identificados como produtos finais da oxidação dos dois poluentes orgânicos modelo, assim como sulfatos para a amoxicilina e cloretos para o diclofenac.

A transformação da amoxicilina e diclofenac em compostos não tóxicos após ozonização fotocatalítica foi confirmada através da realização de ensaios de inibição de crescimento, utilizando duas estirpes diferentes, *Escherichia coli* e *Staphylococcus aureus*. Verificou-se que a amoxicilina perdeu a sua atividade antimicrobiana após 15 min. Por outro lado, nenhum efeito tóxico foi observado para o diclofenac, nem para os seus produtos da degradação.

Os ensaios de inativação permitiram concluir que o processo de ozonização fotolítica é eficaz na desinfeção de água, permitindo a eliminação completa de *Escherichia coli*, após 180 minutos de tratamento.

Neste contexto, o processo de ozonização fotocatalítica revelou ser uma solução eficiente para a degradação de poluentes orgânicos e inativação de microrganismos, de relevância para o desenvolvimento de sistemas para tratamento de águas.

# Nomenclature

| | |
|---|---|
| AMX | Amoxicillin |
| AOPs | Advanced oxidation processes |
| BOD | Biochemical oxygen demand, mg $L^{-1}$ |
| CFU | Colony Forming Units |
| COD | Chemical oxygen demand, mg $L^{-1}$ |
| DAD | Diode array detector |
| DFC | Diclofenac |
| DL | Detection limit |
| DNA | Deoxyribonucleic acid |
| DOC | Dissolved organic carbon, mg $L^{-1}$ |
| HPLC | High performance liquid chromatography |
| IC | Inorganic carbon, mg $L^{-1}$ |
| LA | Luria agar |
| MF | Membrane filter |
| m-FC | Membrane filter – Fecal coliforms |
| NI | Non-identified |
| OD | Optical density |
| STP | Sewage treatment plant |
| SW | Synthetic wastewater |
| TC | Total carbon, mg $L^{-1}$ |
| TOC | Total organic carbon, mg $L^{-1}$ |
| US EPA | United States Environmental Protection Agency |
| UV | Ultraviolet |
| WWTPs | Wastewater treatment plants |

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# 1 Introduction

## 1.1 Overview of the problematic

Water scarcity is an important problem in different regions of the globe, and water reuse practices are of major relevance nowadays. On the other hand, public health is strongly affected by the quality of the drinkable water. A single water source can serve a large number of people, and a bad control of water quality may lead to serious public health problems [1, 2]. All chemical and biological contaminants should be then avoided in natural water courses and it becomes essential a proper water treatment and disinfection, not only for consumption but also for discharge in natural courses. Therefore, it is crucial to develop efficient treatment technologies for municipal wastewater treatment plants (WWTPs).

During the last years increasing concentrations of emerging pollutants have been detected in effluents from municipal WWTPs and, as consequence, in aquatic environments [3]. US EPA directives define emerging pollutants as chemical compounds that are not regulated and whose impact on the environment and human health is poorly understood yet [4]. One of the main causes for the progressive increase of pharmaceutical contaminants in water is the today large production of drugs, ca. 4,000 types in the order of 100 to 200,000 tons annually, over the world [5, 6]. For instance, Madigan et al. [7] reported that more than 500 metric tons of antimicrobial chemotherapeutic agents are manufactured worldwide every year.

The large amount of drugs that is prescribed and consumed by humans or animals, and subsequently excreted by them, are ending in municipal WWTPs where only a small fraction of these drugs is destroyed. In fact, many of them are purposely made to be durable and to remain biologically active with the aim to be efficient in health care, what makes them very difficult to be degraded by the conventional treatments used in WWTPs. In this context, it is considered that effluents from WWTPs are causing major negative impacts on the environment. Figure 1 shows some data identified by Deblonde et al. [4] regarding the removal rate of different classes of compounds in WWTPs. Among them, antiepileptics, contrast media and antibiotics, as well as analgesics and anti-inflammatories (AAI), are the most resistant to the typical treatments.

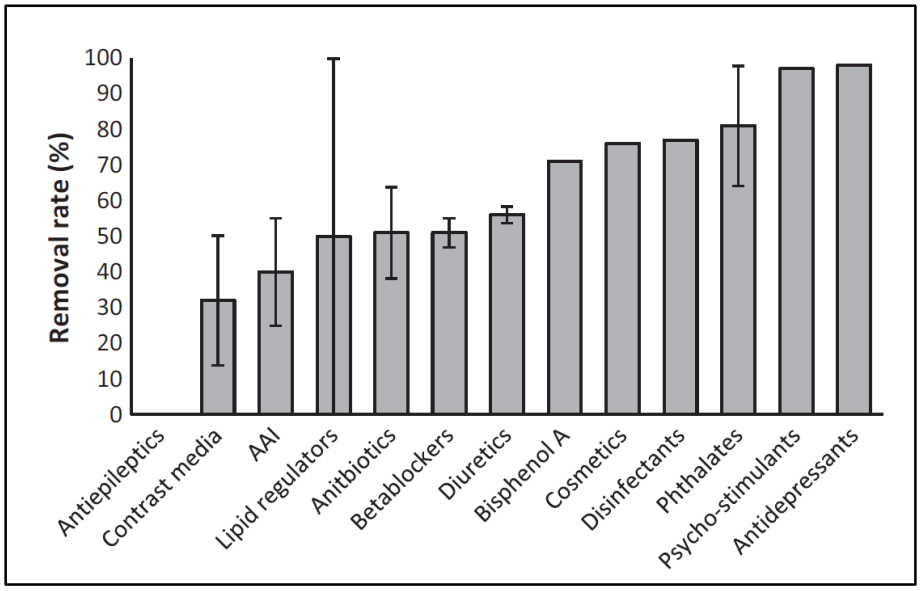



**Figure 1** – Removal rate for each class of compounds calculated from the database from Deblonde et al. [4]

It is of general interest to reduce the emission of these pollutants since they can be very toxic to ecosystems even when appearing at very low concentrations. In addition, organic micropollutants have been detected in drinking water, and even if the impact of this type of pollution on living beings is not yet fully understood, a serious indirect problem is associated with the development of multiresistant microorganisms to antibiotics. Spreading of antibiotic resistance among bacteria may occur if antibiotics (as well as antibiotic resistant bacteria) are not completely eliminated in WWTPs [8, 9].

Some resistance mechanisms were identified by Madigan et al. [7]: (i) the organism may lack the structure an antibiotic inhibits (e.g. mycoplasmas lack a bacterial cell wall and therefore naturally resistant to penicillins); (ii) the organism may be impermeable to the antibiotic (e.g. a high amount of Gram-negative bacteria are impermeable to penicillin G); (iii) the organism may be able to alter the antibiotic to an inactive form (e.g. β-lactamases, an enzyme that cleaves the β-lactam ring of most penicillins); (iv) the target of the antibiotic can be modified by the organism, a resistant biochemical pathway can be established and finally efflux can occur (organism can pump out an antibiotic entering the cell).

The primary principle of a municipal WWTP is to allow water to be subsequently restored on water resources without affecting the environment and ecosystems and its reuse for a wide variety of activities. Therefore, besides removing organic compounds from water,

WWTPs should also reduce the microbial load. There are several factors affecting the efficiency of a disinfection process, such as the nature of microorganisms, type and concentration of the disinfectant agent, contact time, pH, temperature and composition of the water (e.g., chemical compounds). The damage provoked by the disinfectant agent can affect different elements of the cell like the membrane, DNA and others [1, 10]. The legislation regarding water reuse requires to verify the treated water microbiological quality, and the main indicators are the total coliforms, fecal coliforms and *Escherichia coli*. Microorganisms belonging to the group of coliforms are usually used as indicators of microbial contamination, since the majority of them inhabit in the intestinal tract of humans and animals. Therefore, their presence indicates fecal contamination. The coliform group includes a variety of organisms such as *Escherichia coli*.

## 1.2 Advanced oxidation processes

Since secondary treatments cannot remove all existing chemical and biological pollution in WWTPs, tertiary treatments should be applied in order to improve the treatment process. Advanced oxidation processes (AOPs) [3, 5, 11, 12] are seen as alternatives to combat the above mentioned problems, i.e., they are recommended technologies for tertiary treatment in WWTPs (as shown in Figure 2) in order to ensure the safe discharge of municipal WWTPs effluents [13].

AOPs are conceptually based on the generation of highly reactive free radicals, such as the powerful hydroxyl radicals ($OH^{\bullet}$), whose can rapidly oxidize organic compounds in water. The attack of the molecules by the $OH^{\bullet}$ radicals causes a series of oxidation reactions that ideally lead to the mineralization of the organic pollutants [6, 14, 15]. Ozonation, heterogeneous photocatalysis, the Fenton process, wet peroxide oxidation and wet oxidation are the most known AOPs, and some of them can be assisted by active catalysts and/or by hydrogen peroxide [14, 16].

It is also possible to combine different AOPs with the aim to increase the overall treatment efficiency. For instance, some organic compounds can be very difficult to degrade by a process alone, as well as the products resulting from the reaction in one single process can be more toxic than the initial pollutants, while the combination of two processes can be more efficient in the oxidation of both parent and intermediate

compounds [17]. Other important approach is to verify if AOPs can inactivate and eliminate the water microbial community, since this will become of high interest in WWTPs. When performing microbiological analysis, it is necessary to take into account the fact that some of the AOPs can only inactivate these microorganisms, and the damage can be reversible. These processes, especially those where UV radiation or a catalyst are implemented, can be effective on eliminating the bacterial community due to the production of the $OH^{\bullet}$ radicals [2, 7].

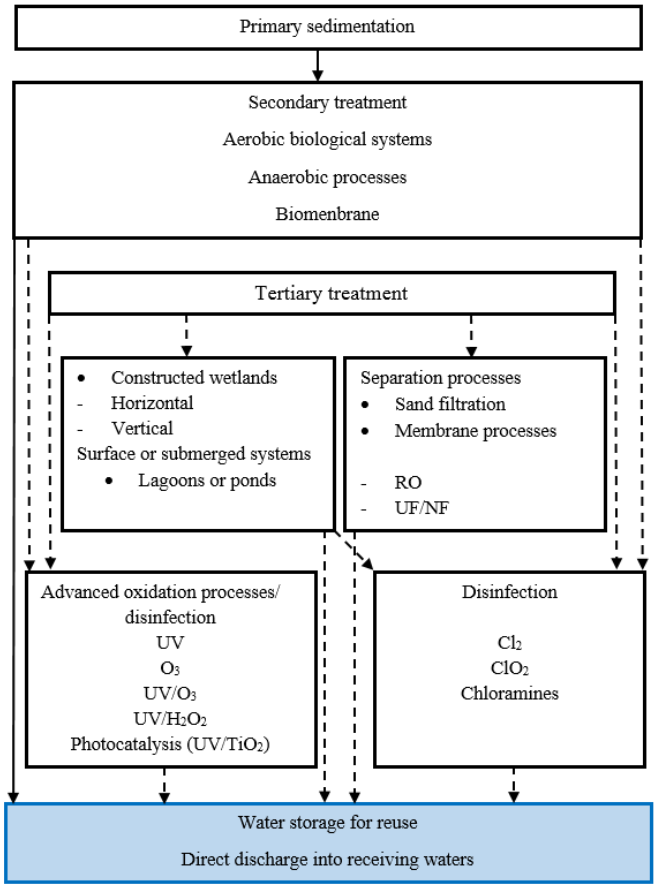



**Figure 2** – Integration of AOPs in the treatment of municipal wastewater form Schaechter et al. [13].

In the present work, single ozonation and photocatalysis were used alone and combined in one single reactor; thus, major relevance will be given to these two AOPs in the following sections.

## 1.3 Photocatalysis

Titanium dioxide ($TiO_2$) is one of the most used photocatalysts due to its high chemical stability, low toxicity, affordable cost and electronic and optical properties. $TiO_2$ is a very powerful photocatalyst when excited under UV or near-UV-vis radiation. Highly reactive surface excited electrons and holes are generated by photons with energy equal or above the band-gap energy of the semiconductor, typically near 3.2 eV for $TiO_2$. These electrons and holes are responsible for the oxidation of the organic compounds by the formation of free radicals (such as $OH^{\bullet}$ and $O_2^{\bullet -}$) or by direct oxidation of the pollutants by photogenerated holes [3, 17, 18].

The reference photocatalyst in literature is a $TiO_2$ material from Evonik Degussa (P25), consisting of two crystalline $TiO_2$ phases (near 80% anatase and 20% rutile). Anatase crystalline form is generally considered more active than rutile alone, but rutile has a lower band-gap energy and can presents some photocatalytic activity under visible radiation (in contrast to anatase). The following reaction can occur at the surface of a $TiO_2$ particle [3, 14, 19, 20]:

$$TiO_2 + h\nu \rightarrow e^- + h^+ \quad (1)$$

$$e^- + O_2 \rightarrow O_2^{\bullet -} \quad (2)$$

$$h^+ + H_2O \rightarrow OH^{\bullet} + H^+ \quad (3)$$

$$e^- + O_2 + H^+ \rightarrow HO_2^{\bullet} \quad (4)$$

$$h^+ + OH^- \rightarrow OH^{\bullet} \quad (5)$$

$$OH^{\bullet} + RH \rightarrow RHOH^{\bullet} \quad (6)$$

$$OH^{\bullet} + RH \rightarrow R^{\bullet} + H_2O \quad (7)$$

$$h^+ + RH \rightarrow RH^{\bullet +} \quad (8)$$

The first step is the generation of excited electrons and holes (Eq. 1). Then, hydroxyl and superoxide radicals are produced on the surface of $TiO_2$ (Eqs. 2–5). In this way the organic compound can be oxidized by the radicals or even by the generated holes (Eqs. 6-8).

## 1.4 Ozonation

Ozone is a strong oxidant with great potential for disinfection. The ozonation process is more efficient than photocatalysis to treat highly contaminated wastewaters. However, the use of ozone alone does not lead to complete mineralization of many organic contaminants in water, since typical oxidation reaction intermediates, such as carboxylic acids, are hardly degraded by ozone. This oxidizing agent has the ability to attack organic compounds directly and selectively at low pH, or can undergo decomposition via a chain reaction mechanism that produces $OH^{\bullet}$ radicals at high pH. Ozone attacks preferentially aromatic molecules and unsaturated bonds. The half-life of molecular ozone depends on several operating parameters, such as pH and temperature [16, 17, 19, 21]. Ozone decomposition proceeds through the following chain reactions (Eqs. 9–13) [16, 22]:

$$O_3 + H_2O \rightarrow 2HO^{\bullet} + O_2 \quad (9)$$

$$O_3 + OH^- \rightarrow O_2^{\bullet -} + HO_2^{\bullet} \quad (10)$$

$$O_3 + OH^{\bullet} \rightarrow O_2 + HO_2^{\bullet} \leftrightarrow O_2^{\bullet -} + H^+ \quad (11)$$

$$O_3 + HO_2^{\bullet} \leftrightarrows 2O_2 + OH^{\bullet} \quad (12)$$

$$2HO_2^{\bullet} \leftrightarrows O_2 + H_2O_2 \quad (13)$$

Due to its structure, molecular ozone can react as a dipole, an electrophilic or nucleophilic agent. Figure 3 shows the two resonance structures of molecular ozone [16].

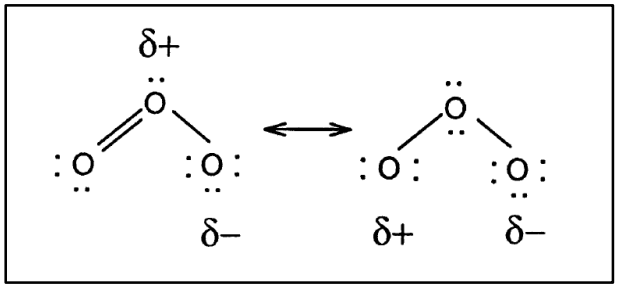



**Figure 3** – Resonance structures of ozone molecule from Kasprzyk-Hordern et al. [16].

## 1.5 Photocatalytic ozonation

According to Sanchez et al. [23], the photocatalytic ozonation process induces the generation of $OH^{\bullet}$ radicals through the formation of an ozonide radical ($O_3^{\bullet -}$) in the adsorption layer of a semiconductor, such as $TiO_2$. Specifically, the $O_3^{\bullet -}$ species (Eq. 14) generated from the reaction of ozone with the semiconductor electrons (Eq. 1) will react with $H^+$ in the solution, forming $HO_3^{\bullet}$ radicals (Eq. 15). These radicals will evolve in order to produce oxygen and $OH^{\bullet}$ radicals (Eq. 16). In this scenario, it must be also considered that $OH^{\bullet}$ radicals can react with ozone (Eq. 17). When ozone is completely consumed, dissolved oxygen can accept the semiconductor conduction band electrons, generating $O_2^{\bullet -}$ (Eq. 18), which in turn can be protonated (Eq. 19). $HO_2^{\bullet}$ radicals can also originate $OH^{\bullet}$ radicals, according to the pathway shown by Eqs. 20–21.

$$O_3 + e^- \rightarrow O_3^{\bullet -} \quad (14)$$

$$O_3^{\bullet -} + H^+ \rightarrow HO_3^{\bullet} \quad (15)$$

$$HO_3^{\bullet} \rightarrow O_2 + OH^{\bullet} \quad (16)$$

$$OH^{\bullet} + O_3 \rightarrow O_2 + HO_2^{\bullet} \quad (17)$$

$$O_2 + e^- \rightarrow O_2^{\bullet -} \quad (18)$$

$$O_2^{\bullet -} + H^+ \rightarrow HO_2^{\bullet} \quad (19)$$

$$2HO_2^{\bullet} \rightarrow H_2O_2 + O_2 \quad (20)$$

$$H_2O_2 + O_2^{\bullet -} \rightarrow OH^{\bullet} + OH^- + O_2 \quad (21)$$

Another possible reaction pathway was proposed by Kopf et al. [24]. These authors suggested that the photocatalytic ozonation reactions are initiated by an electron transfer from $TiO_2$ to $O_2$ (Eqs. 1-3), and then $O_2^{\bullet -}$ reacts with ozone (Eq. 22), giving place to Eqs. 15-16, as well as to Eqs. 23-24.

$$O_3 + O_2^{\bullet -} \rightarrow O_3^{\bullet -} + O_2 \quad (22)$$

$$OH^{\bullet} + R - H \rightarrow R^{\bullet} + H_2O \quad (23)$$

$$OH^{\bullet} + R \rightarrow R^{\bullet} - OH \quad (24)$$

There are some factors that can affect photocatalytic ozonation process, some of them similar to those affecting the single processes, such as the ozone dosage, pH and nature of the photocatalyst. The increase of ozone dosage can increase the TOC removal, but from an economic point of view low ozone dosages are economically more attractive [25]. The pH of the effluent has a stronger influence on the single ozonation reaction, and can change the degradation pathways and the kinetics in the reaction process. This can also occurs in the photocatalytic ozonation process, depending on the pollutants involved [26, 27]. Regarding the nature of the catalyst, different $TiO_2$ crystalline forms, particle sizes, among others, have different photoactivity. In addition, when the organic compounds are not completely degraded, the partial oxidation may produce by products that are more toxic than the parent pollutants [17, 28].

In fact, the scientific community interest on the identification of reaction intermediates has increased from an ecotoxicological point of view. The adverse effects of substances on different organisms can be studied by using toxicity bioassays. In this way, it is possible to detect the combined toxicity of mixtures of known and unknown compounds (with similar inhibitory mode of action), while by common chemical analysis it is only possible to quantify targeted chemical compounds without identification of their effect on the environment. Microorganisms, plants, algae, invertebrates and fish are the principal test organisms used in the toxicity bioassays [29].

## 1.6 Objectives

The aim of this work was to study the degradation of organic pollutants and the inactivation of microorganisms, through the integration of one or more advanced chemical treatment processes in a single reactor. The specific objectives of this work were:

- Study the effect of AOPs on AMX and DFC degradation in different matrices, ultrapure water and synthetic wastewater;
- Verify the effect of advanced oxidation processes in the inactivation of microorganisms present in water;

- Growth inhibition tests for samples containing AMX and DFC in order to verify their toxicity properties and if during the advanced oxidation process compounds even more toxic than the original organic compounds are formed.

# 2 State of the art

Since the progressive increase of pharmaceutical compounds in water is a major problem, their removal from water has been studied by several authors. Table 1 shows some recent works related with AMX and DFC removal by using AOPs. By the analysis of the different works was possible to verify that single ozonation and photolytic oxidation of AMX and DFC leads to high degradation rates but lower TOC removal. In most of the cases, no significant degradation rates were verified for photolysis. The most promising advanced oxidation process for the complete degradation of the organic compounds and high TOC removals is photocatalytic ozonation.

**Table 1 –** Compilation of recent works of AMX and DFC removal by AOPs in water, including the AOPs tested, the degraded compound, the catalyst used, operational condition and the results achieved.

| Process | Compound, catalyst and operational conditions | Results and comments | References |
|---|---|---|---|
| UV/$TiO_2$<br>UV/$H_2O_2$/$TiO_2$ | AMX (104 mg $L^{-1}$)<br>$TiO_2$ powder (0,5-2,0 g $L^{-1}$)<br>6 W UV-A (365 nm) | No significant degradation (3.9%) occurred by 300-min UV-A irradiation.<br>Addition of $H_2O_2$ lead to DOC removal, $NO_3^-$, $NH_3$ and $SO_4^{2-}$ formation. | [20] |
| UV-C<br>UV/$H_2O_2$ | AMX (10 μM)<br>Low pressure Hg arc-UV lamp (254 nm) | <10% of TOC removal after 80 min of irradiation for photolysis;<br>More than 99% of the AMX was degraded in 20 min with addition of 10 mM $H_2O_2$ and 50% TOC removal after 80 min. | [30] |
| UV-A/$TiO_2$ | AMX (2.5 – 30 mg/L)<br>$TiO_2$ Degussa P25 (100-750 mg $L^{-1}$)<br>9 W lamp UV-A (350-400 nm)<br>Ultrapure water and secondary treated effluent. | Complete AMX and 93% mineralization for Degussa P25 could be achieved after 25 and 90 min of reaction, respectively at 10 mg $L^{-1}$ AMX and 250 mg $L^{-1}$ titania.<br>Degradation in treated effluent was partly impeded compared to pure water due to the inherent presence of organic and inorganic constituents that compete for hydroxyl radicals. | [31] |
| UV/ZnO | AMX (104 mg $L^{-1}$)<br>ZnO (0.5 g $L^{-1}$)<br>6 W UV lamp (365 nm) | pH has great effect on AMX<br>23.9% COD removal<br>9.7% DOC removal<br>Complete degradation of AMX | [32] |

**Table 1** – (Continued)

| Process | Compound, catalyst and operational conditions | Results and comments | References |
|---|---|---|---|
| UV/$TiO_2$ | AMX (1–100 mg $L^{-1}$)<br>$TiO_2$ powder (1 g $L^{-1}$)<br>15 W low pressure luminescent mercury UV-lamp (365 nm) | Maximum efficiency in neutral solutions;<br>The efficiency increases with growing concentration of AMX achieving maximum at 50 mg $L^{-1}$;<br>Reaction products suggest possible reaction pathways. | [33] |
| UV/$TiO_2$<br>UV/($Sn/TiO_2$) | AMX Trihydrate (20 mg $L^{-1}$)<br>$Sn/TiO_2$ (0 – 3 mol %)<br>15 W (UV-C) mercury lamp (254 nm) | $K_{AMX}$ = 0.56 mg $L^{-1}$<br>$K_c$ = 1.86 mg $L^{-1}$ | [3] |
| UV<br>$O_3$<br>UV/$TiO_2$<br>UV/$O_3$<br>UV/$O_3$/$TiO_2$ | AMX (1 μM)<br>$TiO_2$ Degussa P25<br>(0.001 and 0.005 g $L^{-1}$)<br>Ozone flow rate – 16 mg $h^{-1}$<br>15 W low-pressure mercury lamp (264 nm) | Pseudo-first-order rate constant<br>Photolysis – $k$p = 0.109 $min^{-1}$;<br>Photocatalysis (0.001 g/L) – $k_T$ = 0.114 $min^{-1}$;<br>Photocatalysis (0.005 g/L) – $k_T$ = 0.347 $min^{-1}$;<br>Single ozonation – $kO_3$ = 0.559 $min^{-1}$;<br>Photolytic ozonation - $k_T$ = 1.172 $min^{-1}$<br>Photocatalytic ozonation (0.001) - $k_T$ = 1.203 $min^{-1}$ | [34] |
| UV/$TiO_2$ | AMX (20-40 mg $L^{-1}$)<br>$TiO_2$ Degussa P25 (0.5 g $L^{-1}$)<br>Solar pilot plant<br>Lab-scale photoreactor – radiation intensity – 44 $W_{uv}$ $m^{-2}$ (250 to 400 nm) | Photolysis – ineffective;<br>3.1 $kJ_{uv}$ $L^{-1}$ was sufficient to fully degrade 20 mg $L^{-1}$ AMX and 61% removal of DOC in the presence of photocatalyst and sunlight;<br>Antibacterial activity of the solution was reduced (AMX - 40mg $L^{-1}$) after elimination of AMX;<br>After 11.7 $kJ_{uv}$ $L^{-1}$ DOC decrease 71%<br>30% of AMX nitrogen was converted to ammonium and all sulfur into sulfate | [35] |
| UV | AMX (1 mg $L^{-1}$)<br>Wide spectrum 250 W lamp<br>UV dose – $0.42\times10^4$ μW s $cm^{-2}$ | Low solar photodegradation<br>Pseudo first order kinetics – k = 0.0335 min-1;<br>$T_{1/2}$ = 20 min | [36] |
| $O_3$ | AMX ($5.0\times10^{-4}$ M)<br>Flow rate – 36 L $h^{-1}$<br>Ozonized oxygen stream of 2% by volume | AMX – 90% degradation<br>TOC removal – 18.2% | [11] |

**Table 1** – (Continued)

| Process | Compound, catalyst and operational conditions | Results and comments | References |
|---|---|---|---|
| $O_3$ | AMX (10 μM)<br>Mass flow rate of 16 mg $h^{-1}$ of ozone; | Determination of the apparent rate constants for the reactions between ozone and AMX. Values ranged between $2.31\times 10^3$ and $1.21\times10^7$ $M^{-1}$ $s^{-1}$. | [37] |
| $UV/O_3/TiO_2$ | DFC (30-80 mg $L^{-1}$)<br>$TiO_2$ Degussa P25 (0.5-2.5 g $L^{-1}$)<br>Ultrapure water and urban wastewater treatment<br>High pressure mercury lamp (313 nm)<br>Gas flow rate – 30-50 L $h^{-1}$<br>Ozone concentration – 5-30 mg $L^{-1}$ | Photocatalytic ozonation – 90% TOC removal after 15 min of reaction. Lowest ozone consumption per mg TOC consumed.<br>Photocatalytic oxidation processes gave the lowest ecotoxicity.<br>No differences observed in both DFC and TOC removal, when using real wastewater, compared to those observed in ultrapure water. | [38] |
| $O_3$<br>$O_2/UV\text{-}A/TiO_2$<br>$O_3/UV\text{-}A/TiO_2$<br>$UV\text{-}A/TiO_2$ | DFC ($1.0\times10^{-4}$ mol $L^{-1}$)<br>$TiO_2$ Degussa P25 (1.5 g $L^{-1}$)<br>11.6 W High-pressure mercury vapor lamp 313 nm<br>Inlet ozone gas concentration – 10 mg $L^{-1}$<br>Gas flow rate – 30 L/h | Fast and complete elimination of DFC for single ozonation but slow TOC removals rates (50% mineralization).<br>Complete removal of DFC and higher TOC removal (80% mineralization) for the photocatalytic processes | [39] |
| $O_3$ | DFC (1.3 μg $L^{-1}$)<br>STP effluent (2 $m^3$ $h^{-1}$)<br>Inlet ozone gas concentration – 15 mg $L^{-1}$ | >96% DFC removal | [40] |
| $O_3$<br>$H_2O_2/UV$ | DFC ($5.0\times10^{-4}$ M)<br>17 W low-pressure mercury monochromatic lamp (254 nm)<br>Gas flow rate – 36 L $h^{-1}$ | Ozonation – 32% mineralization and 95% chlorine conversion to chloride;<br>$H_2O_2/UV$ – 39% mineralization and 52% chlorine conversion to chloride;<br>No mineralization with UV irradiation alone;<br>Identification of intermediates. | [41] |
| $O_3$ | DFC (200 mg $L^{-1}$) | Ozone dosage of 0.15 g $L^{-1}$ after 20 min of reaction was enough to achieve 95% DFC removal. At an ozone dosage of 0.172 g $L^{-1}$ DFC concentration was lower than 2 mg $L^{-1}$;<br>Ozone contributes slightly to TOC removal;<br>Single ozonation is efficient to remove DFC, but not to mineralize the by-products;<br>TOC removal increase until 20% removal; | [49] |

**Table 1** – (Continued)

| Process | Compound, catalyst and operational conditions | Results and comments | References |
|---|---|---|---|
| $O_3$<br>UV<br>$UV/TiO_2$<br>$UV/O_3/TiO_2$ | DFC (200 μg $L^{-1}$)<br>Catalyzed reactions with 2.8 mg $L^{-1}$ Fe(III) or 150 mg $L^{-1}$ $Fe_3O_4$<br>Flow rate – 35 L $h^{-1}$<br>Inlet ozone gas concentration – 13 mg $L^{-1}$<br>15 W tubular black light (BL) lamps (HQ Power Lamp 15TBL) (365 nm) | Initial compound completely degraded after 10 min with any ozone AOP. No degradation with photolysis.<br>35% TOC removal for photocatalytic ozonation while just 13% for single ozonation, after 30 min of reaction. | [42] |
| Sequential (UV) and $O_3$ | DFC (Different concentrations for each month)<br>UV dosage – 60 mJ $(cm^2)^{-1}$<br>Ozone dosage – 5 mg $L^{-1}$<br>Contact time in the ozone chamber – 15 min | >80% DFC removal;<br>Removal efficiencies were highly dependent on the reaction rate constants with molecular ozone;<br>The UV unit had marginal effect to removal of all the pharmaceutical compounds tested except for DFC. | [43] |
| Photolysis<br>$UV/TiO_2$ | DFC (50 mg $L^{-1}$)<br>$TiO_2$ Degussa P25 (0.2 g $L^{-1}$)<br>Parabolic Collector solar pilot plant | Photolysis: 68% DFC removal after 32 min for demineralized water and 85% removal after 62 min for standard freshwater. Mineralization never occurred.<br>$TiO_2$ photocatalysis: Total decomposition after 200 min and DOC oxidation after 100 min. Chloride and ammonium formation. | [44] |
| $UV/TiO_2$ | DFC (15 mg $L^{-1}$)<br>$TiO_2$ Degussa P25 (200 mg $L^{-1}$);<br>Xenon arc lamp (1500 W) with special glass filters (wavelengths below 290 nm);<br>Average irradiation intensity of 750 W $m^{-2}$. | Complete DFC degradation after 1h and 99% TOC removal after 2 h. Formation of chlorides, ammonium and nitrate ions. | [45] |
| $UV/TiO_2$ | DFC (280 (±50) ng $L^{-1}$);<br>$TiO_2$ Degussa P25 (50 mg $L^{-1}$);<br>Energy per order (EEO) – 0.21 (±0.040) kW h $m^{-3}$.<br>Photocatalytic reactor membrane pilot system; | Removal constant – 8.2 (±1.6) $m^3\,kW^{-1}\,h^{-1}$ | [46] |

**Table 1** – (Continued)

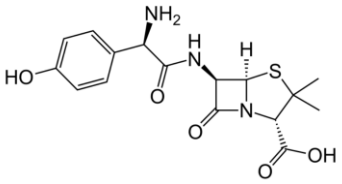
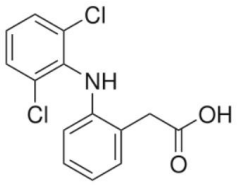
| Process | Compound, catalyst and operational conditions | Results and comments | References |
|---|---|---|---|
| UV<br>UV/$TiO_2$ | DFC (200 mg $L^{-1}$)<br>Different operational conditions – catalyst load, temperature and dissolved oxygen concentration;<br>Solarbox 1kW Xe-OP lamp with a photon flux equal to 6.9 μEinsteins/s (290-400 nm) | In photolysis was verified 75% DFC degradation and 25% TOC removal after 2h.<br>Maximum DFC degradation was observed at a $TiO_2$ loading of 0.1 g $L^{-1}$.<br>TOC removal increased with the $TiO_2$ loading. | [47] |
| $O_3$ | DFC (4, 40 and 80 mg $L^{-1}$)<br>Ozone flow rate – 31 g $h^{-1}$<br>Ozone concentration – 15 mg $L^{-1}$ | Maximum TOC reduction achieved was 30% for 80 mg $L^{-1}$ after 40 min;<br>Almost complete DFC removal after 40 minutes;<br>The removal rate significantly increased when the DFC initial concentration was reduced. | [48] |

# 3 Experimental set-up and analytical methods

## 3.1 Tested emerging pollutants

In this work two emerging contaminants with different structures, amoxicillin (AMX) and diclofenac (DFC) were tested as model chemical pollutants in aqueous solutions. Amoxicillin ((2S,5R,6R)-6-[[(2R)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid) is a penicillin antibiotic used to treat many different types of infections caused by a wide range of Gram-positive and Gram-negative bacteria in both humans and animals and has been found to be more effective against Gram-positive than Gram-negative microorganisms. In the past decades, amoxicillin was used to treat infections of the middle ear, tonsils, throat, larynx, pharynx, bronchi, lungs, urinary tract, skin and to treat gonorrhea [11, 50]. Amoxicillin was provided by Fluka. Diclofenac ([2-(2,6-dichlorophenylamino)-phenyl]-acetic acid) is a widely used anti-inflammatory non-steroidal drug with great resistance to biodegradation. Diclofenac is mostly used as its sodium salt in medical care as analgesic, antiarthritic and antirheumatic [39, 51, 52]. Diclofenac was provided by Cayman Chemical Company.

**Table 2 –** Class, chemical structure and maximum absorption wavelength ($\lambda_{max}$) of the emerging pollutants tested.

| Class | Model pollutant and chemical structure | $\lambda_{max}$ (nm) |
|---|---|---|
| **Penicillins (β-lactam antibiotics)** | Amoxicillin  | 200 |
| **Benzene and Substituted Derivatives** | Diclofenac  | 200 |

## 3.2 Experimental set up and procedure

Four different AOPs were studied in the present work, namely single ozonation, photolysis (i.e., in the absence of a catalyst), photolytic ozonation and photocatalytic ozonation. The experiments were performed in a lab-scale reactor shown in Figure 4. Ozone was produced from pure oxygen in a BMT 802X ozone generator. The reactor was equipped with a Heraeus TQ 150 medium-pressure mercury vapor lamp located axially and held in a quartz immersion tube with water recirculation to maintain a room temperature in the experiments (near UV-vis radiation with dominant emission lines at 366, 436 and 546 nm).

In a typical run, the reactor was filled with 250 mL of the pollutant solution with an initial concentration of 0.1 mM, regardless the pollutant used, and magnetically stirred at 400 rpm. This concentration is significantly higher than those found in typical WWTPs, but by this way it was possible to analyze the concentration of the organic compounds in the experiments without implementing more sophisticated, expensive and time-consuming analytical techniques.

In experiments with ozone, a constant ozone flow rate (150 $Ncm^3\ min^{-1}$) and a constant inlet ozone concentration (50 g $Nm^{-3}$) were used. The concentration of ozone in the gas phase was monitored with a BMT 964 ozone analyzer. The ozone leaving the reactor in the gas phase was removed in gas washing bottles filled with potassium iodide solution. In photolysis, the ozone containing stream was replaced by an oxygen stream, at the same flow rate (150 $Ncm^3\ min^{-1}$), and in this case the lamp was turned on. Photocatalytic experiments were similar but in this case the reference $TiO_2$ photocatalyst Evonik Degussa P25 was used in the powder form (catalyst load of 0.5 g $L^{-1}$). In this case all samples were centrifuged with a WWR MicroStar 12 apparatus to remove any possible suspended particles. The photolytic ozonation experiments were performed by using the conditions employed for photolysis, but replacing oxygen by ozone. In all experiments samples at defined intervals were taken from the reactor through a syringe for analysis.

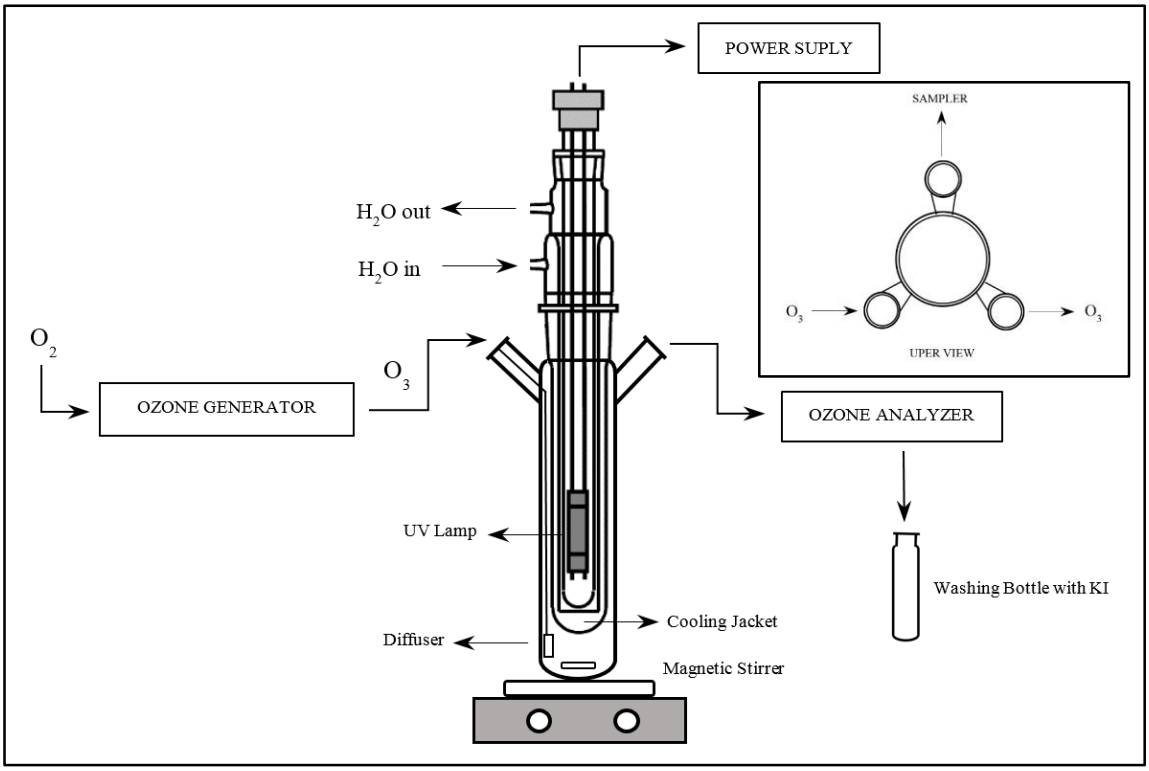



**Figure 4** – Schematic representation of the lab-scale reactor (inset: upper view).

## 3.3 Synthetic wastewater

A synthetic wastewater was prepared in order to simulate the real composition of an effluent from a municipal WWTP (Table 3).

**Table 3** – Composition of the synthetic wastewater.

| Component | mg $L^{-1}$ |
|---|---|
| $(NH_4)_2SO_4$ | 15 |
| Triptone | 50 |
| Meat extract | 50 |
| Yeast extract | 7.5 |
| Urea | 7.5 |
| $K_2HPO_4$ | 10 |
| $CaCl_2.2H_2O$ | 1 |
| $MgSO_4.7H_2O$ | 1 |

The composition of the synthetic wastewater was selected having into account reference values reported in the literature [53]. The aim was to treat this synthetic wastewater, containing also the model chemical and microbiological pollutants. The biochemical oxygen demand (BOD) and the chemical oxygen demand (COD) of the synthetic

wastewater was determined to assure that it meets the requirements established in legislation for discharge of effluents from WWTPs in water courses.

Initially were prepared stock solutions for each of the components 100× concentrated, using distillated water. Each stock were posteriorly autoclaved. The stock solutions were stored in a refrigerator at 4 ºC. The synthetic wastewater was always freshly prepared by the addition of a determined volume of each component from the stocks in order to achieve the pretended concentrations. For the study of the effectiveness of photolytic ozonation on bacterial inactivation 2 L of synthetic wastewater was prepared in a sterile glass flask, by the addition of 20 mL of each previously prepared stock and the remaining volume was completed with sterile distilled water, while for the photolytic ozonation of AMX in a synthetic wastewater, was prepared 1 L and added 10 mL of each stock, and then the remaining volume was completed with ultrapure water.

## 3.4 Effectiveness of photolytic ozonation on bacterial inactivation

To verify the effect of photolytic ozonation on microorganisms present in the wastewater, the synthetic wastewater was inoculated with a suspension of *Escherichia coli* DSM 1103 at an initial density of 1 x $10^3$ CFU $mL^{-1}$.

To obtain the *E. coli* suspension, biomass previously grown overnight on Plate Count Agar plates at 30 ºC was used to prepare a suspension in a test tube with 9 mL of sterile saline solution (0.85 % NaCl (w/v)). Then, the cell density of the suspension was adjusted to 1 x $10^8$ CFU $mL^{-1}$ through a calibration curve (CFU $mL^{-1}$ = 1 x $10^9$ OD (610 nm)) of optical density versus number of viable counts (CFU $mL^{-1}$), which correspond to an OD equal to 0.1 at 610 nm. Then, a 10-fold dilution was made, in a test tube with 9 mL of sterile saline, in order to get a 1 x $10^7$ CFU $mL^{-1}$ cell density suspension.

Primarily 2 L of inoculated synthetic wastewater were freshly prepared in a glass flask, by the addition of 1838 mL of sterile distillated water, 20 mL of each previously prepared component stock and 200 μL from the cell suspension in order to obtain an initial cell density approximately equal to 1 x $10^3$ CFU $mL^{-1}$.

The inoculated water was divided in two samples, the first one with the initial inoculated water (1 L) not treated by AOPs and the second one with initial inoculated water (1 L) to

be treated by photolytic ozonation, into sterile 1 L glass flasks. The first sample will be the positive control. For the initial inoculated water without treatment two dilutions were made, 10-fold ($10^{-1}$) and 100-fold ($10^{-2}$). Then the same sample, after 3 h without treatment, was diluted, 100-fold ($10^{-2}$) and 1000-fold ($10^{-3}$). Finally, the inoculated water treated by photocatalytic ozonation was diluted 10-fold ($10^{-1}$), 100-fold ($10^{-2}$) and 1000-fold ($10^{-3}$).

The membrane filter (MF) method was used to count the viable organisms remaining after treatment. The viable cells are those that are able to divide and form offspring, and in most cell-counting situations these are the cells we are interested in. In the viable counting procedure it is assumed that each viable cell can grow and divide to yield one colony, so, colony and cell numbers are proportional [7].

The MF method permits the deposition of cells from liquid on the upper surface of the membrane, and it is possible, with the correct dilution, to grow individual and discrete colonies when the filter is placed on a suitable nutrient surface, for example, agar medium. When the microbial density is too high some cells may not form colonies and some colonies may fuse, but on the other hand if the number of colonies is too small, the calculated count will not have statistical significance. Because a colony-forming unit may contain one or more cells, data are often expressed as the number of colony-forming units (CFU) obtained. This method can be subject to rather large errors, for example, pipetting inconsistencies, sample not homogenized, insufficient mixing and other factors. So it is important to be careful in sample preparation and pipetting and to replicate plates of key dilutions. With this technic it is possible, from a single sample count, to determine the total number of organisms using a non-selective medium and a selective one to count a particular type of organism [7].

After the necessary dilutions in sterile saline solution, in order to be possible to count the viable organisms, the samples were filtered. The samples from the initial inoculated water without treatment were filtered in the initial time and after 3 h. The inoculated water submitted to photolytic ozonation was filtered after 3 h of treatment. In order to confirm if the treatment just inhibited the growth for a short period of time, filtrations were also made 120 and 180 hours after treatment for both treated and not treated inoculated waters. During this time the samples were stored in an incubator at 37 ºC.

Then the sterile filter funnel was placed in the vacuum system. The sterile filter membrane (0.45 μm porosity and 47 mm diameter) was removed from the case, and with sterile forceps (dip forceps in alcohol and pass through a flame using a Bunsen burner) was placed in the system. Then, with the faucet closed approximately 20 mL of sterile saline solution and the desire volume of sample were added. For the initial inoculated water (initial time) and not treated inoculated water (after 3 h without treatment), 1 ml of each dilution was filtered. For the treated inoculated water 1 mL of each dilution and 1, 10 and 100 ml with no dilution, were filtered. After filtration the funnel was washed with sterile saline solution. Then, the membrane was removed using sterile forceps and placed on a non-selective and a selective medium.

The two mediums used were Luria Agar (LA) and membrane filter – fecal coliforms (m-FC). Duplicates were always performed for each sample and dilution. The cultures were incubated at 37 ºC during 24 hours. In the next day the typical CFUs in each medium were counted. The experiment scheme is represented in Figures 5 and 6.

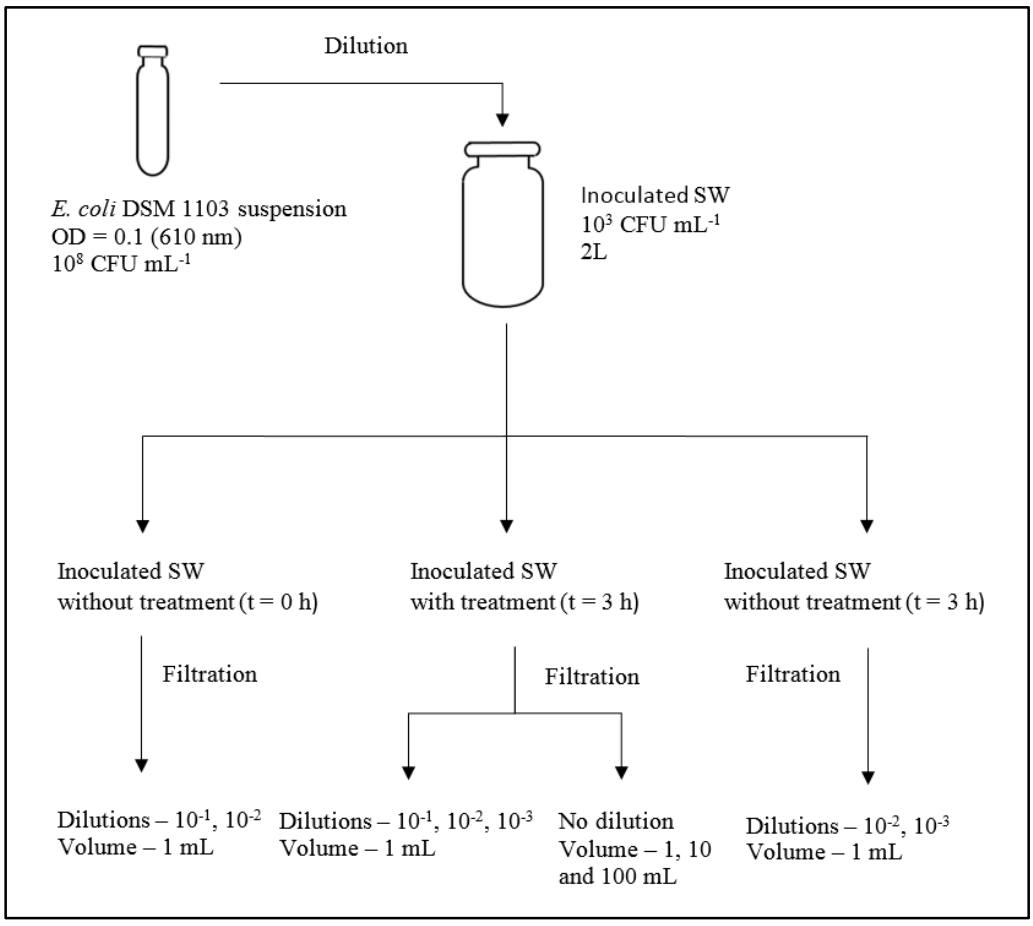



**Figure 5** – Experiment scheme of the assay to verify the effect of advanced oxidation processes in microorganism present in the wastewater.

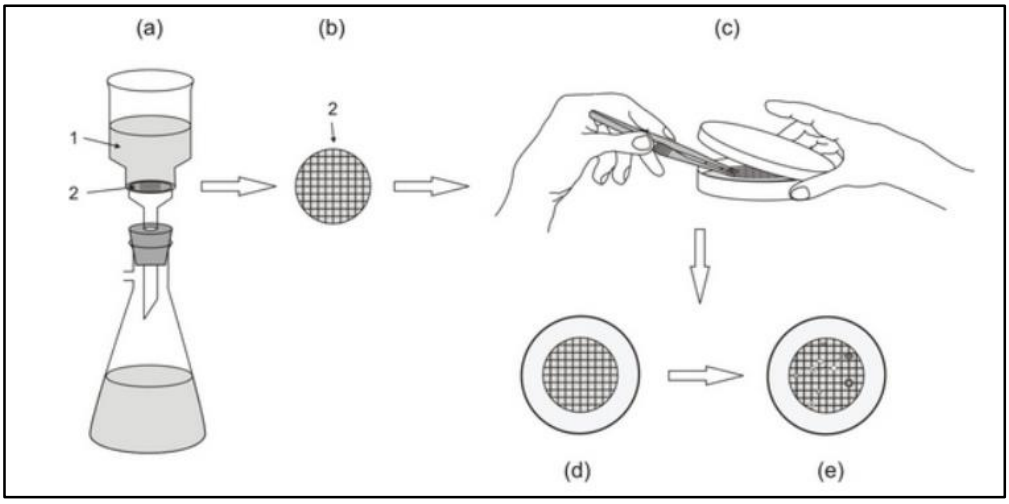



**Figure 6** – Membrane filter method. (a) Filtration, sample (1) and membrane filter (2). (b-c) filter is placed onto a Petri plate using sterile forceps. (d-e) after adequate incubation time, CFU values can be counted from Romsics et al. [54].

## 3.5 Growth inhibition assay for samples containing AMX and DFC

In the second set of microbiological experiments, growth inhibition tests for samples containing AMX and DFC were performed. The initial concentration of AMX and DFC in solution before treatment was 0.1 mM. The samples were collected from the reactor through a syringe 15, 30, 60 and 120 min after photocatalytic ozonation, previously sterilized by filtration using 0.2 µm syringe-filters (Ø 25 mm), to guarantee that only the test strains would growth in the medium, were stored at 4 ºC for no more than 24 h.

Growth inhibition assays were carried out in 96-well microtiter plates using a *Synergy HT Multi-Mode Microplate Reader* (Biotek Instruments, USA) at 37 °C and continuous shaking at medium speed. *Escherichia coli* (DSM 1103) and *Staphylococcus aureus* (DSM 1104) were used as test strains. Absorbance was determined at 610 nm every 30 min (or less).

Each plate included, in quadruplicate, a blank (cell-free medium), more than one in case of cross contamination, a positive control containing the test strain and medium without antibiotic and/or degradation products in order to determine the maximum growth rate that the pure culture can achieve in these conditions, a negative control containing the test strain, medium and AMX or DFC, which corresponds to the initial time of the treatment and finally samples containing the test strain, medium and the samples collected for the different times of reaction.

Each well contains up to 200 µL of sample, since a higher volume can compromise the growth of the test strains, because it will leave no air available for the metabolism. Each well was always supplemented with 2 g $L^{-1}$ of sterile yeast extract.

*E. coli* and *S. aureus* were grown on PCA plates, at 37 °C for ~15 h prior to the assay. Cell suspensions were prepared on 0.85 % (w/v) NaCl with an $OD_{610}$ of 0.8. Each well, except for the blank, were supplemented with 20 µL of this suspension in order to obtain an initial $OD_{610}$ of 0.08 in each.

Specific growth rate (µ, $h^{-1}$) and biomass yield (Y) were calculated, for each well, by adjusting the experimental data of the exponential growth phase to a first-order kinetics model and by the difference between the maximum DO achieved and the initial DO, respectively. Then the values were normalized by the positive control.

## 3.6 Analytical methods

### 3.6.1 Contaminants concentration

The concentrations of AMX, DFC and respective by-products were determined by High Performance Liquid Chromatography (HPLC) using a Hitachi Elite Lachrom HPLC apparatus equipped with a diode array detector (DAD). An YMC Hydrosphere C18 column (250 mm × 4.6 mm) was used in the case of AMX and DCF, working at room temperature under isocratic elution at a flow rate of 1 mL $min^{-1}$. The mobile phase consisted of a 95% phosphate buffer solution (pH = 2.8) and 5% acetonitrile ($CH_3CN$) for AMX and 30% phosphate buffer solution (pH = 2.8) with 70% methanol ($CH_3OH$) for DFC. The concentration of both oxalic and oxamic acids (reaction intermediates) was also determined by HPLC, in this case using an Altech AO-1000 column working at room temperature under isocratic elution with 5 mM $H_2SO_4$ at a flow rate of 0.5 mL $min^{-1}$. The calibration curves for AMX, DFC, oxalic and oxamic acid are presented in Figures A1-A4, Appendix A.

### 3.6.2 Total Organic Carbon

The total organic carbon (TOC) quantifies the organic matter in the solution. By analyzing the TOC evolution it is possible to verify the degree of mineralization achieved by a

particular treatment. TOC was determined in a Shimadzu TOC-5000A analyzer. The analyzer provides the values of total carbon (TC) and inorganic carbon (IC), and the TOC is determined by the difference between TC and IC.

### 3.6.3 Chemical oxygen demand (COD)

Chemical oxygen demand (COD) is defined as the amount of a specified oxidant that reacts with the sample under controlled conditions. The quantity of oxidant consumed is expressed in terms of its oxygen equivalence. COD is often used as a measurement of pollutants in wastewater and natural waters. For the COD determination, the closed reflux method was selected accordingly to the procedure indicated in "Section 5220D. Closed Reflux, Colorimetric Method" from Standard Methods [55].

### 3.6.4 Biochemical oxygen demand (BOD)

The biochemical oxygen demand (BOD) determination is a test that measures the molecular oxygen utilized during a specified incubation period for the biochemical degradation of organic matter and the oxygen used to oxidize reduced forms of nitrogen. The samples were diluted before incubation to bring the oxygen demand and supply into appropriate balance. A solution of nutrients and an inoculum were prepared. The BOD was determined according to the procedure indicated in "Section 5210 B. 5-DAY BOD Test" from Standard Methods [55].

### 3.6.5 Ions concentration

The concentration of ammonium, nitrates, nitrites, sulfates and chlorides was determined by ionic chromatography using a Metrohm 881 Crompaed IC Pro apparatus. A Metrosep C4 Cationic Change Column (250 mm × 4.01 mm) was used for quantification of ammonium and a Metrosep A Supp 7 Anionic Change Column (250 mm × 4.01 mm) for quantification of nitrates, nitrites, sulfates and chlorides. The respective calibration curves are shown in Figures A5-A9, Appendix A.

### 3.6.6 pH

The pH values were measured in WTW InoLab.

# 4 Results and discussion

Initially, three experiments were performed, single ozonation, photolysis, and photolytic ozonation. The interest of performing these experiments separately consisted in verify the performance of each process individually. Subsequently, two AOPs were combined, photocatalytic oxidation and ozonation (i.e. photocatalytic ozonation), in this case using Degussa P25 $TiO_2$, the reference photocatalyst in literature.

In all the experiments mentioned above, samples were systematically taken and different analysis were performed in order to verify if the compounds were degraded and which possible by-products were formed. The concentration of the model pollutants (AMX and DCF) in solution, the concentration of ions, the concentration of some organic by products (oxalic and oxamic acids) and the total organic carbon (TOC) content were analyzed and registered throughout these experiments. The following sections show the effect of the AOPs on each of these parameters.

## 4.1 Effect of various AOPs studied in AMX and DFC concentration

### 4.1.1 Amoxicillin (AMX)

It was found that AMX was degraded very fast in the three experiments where ozone was used. For single ozonation, AMX was degraded in 10 min, whereas for photolytic ozonation and photocatalytic ozonation ca. 5 min were needed to completely remove AMX from the aqueous solution. Andreozzi et al. [11] concluded that the ozone attack is mainly directed towards the phenolic ring of AMX, leading to the formation of hydroxyderivative intermediates.

Even if the degradation of AMX by single ozonation was quite fast, the TOC removal was only ca. 18% at the employed conditions (ozone flow rate of 36 L $h^{-1}$ and an initial AMX concentration of $5.0\times10^{-4}$ M). Therefore, single ozonation was a good process to degrade AMX, but could not degrade efficiently the respective reaction by-products. As explained above, the solution pH is generally considered one of the most important parameters in the single ozonation process [56]. The hydroxide ions promote ozone decomposition into $HO^{\bullet}$ radicals at high pH values, improving, in this way, the efficiency

of the process. However, ozonolysis (direct attack by molecular ozone) is expected to be the main degradation mechanism of the organic compounds in this work, instead of the oxidation through $HO^{\bullet}$ radicals, due to the acidic pH (~ 4) of the aqueous solution. Although ozone is a powerful oxidant, it reacts slowly with some organic compounds and, thus, single ozonation does not cause the complete oxidation of many organic compounds, leading to the accumulation of reaction intermediates, such as carboxylic acids and carbonyl compounds, that cannot be easily oxidized by molecular ozone [17]. Therefore, AMX is easily degraded at the operating conditions employed, but refractory reaction by-products are formed.

Direct photolysis is normally referred to the absorption of UVC photons (254 nm) by the organic compounds and, thus, is not truly considered an AOP having into account that hydroxyl radicals are not generated. In the present work the irradiation source consisted in a Heraeus TQ 150 medium-pressure mercury vapor lamp ($\lambda_{exc}$ = 254, 313, 366, 436 and 546 nm), but the DURAN® glass cooling jacket cut the lower wavelengths, only near-UV to visible light irradiation reaching the AMX aqueous solution (i.e. 366, 436 and 546 nm). For the experiment of photolysis at these conditions, AMX degradation was not observed (i.e., AMX concentration remains nearly the same during all the experiment, < 5% degradation). This result is clearly justified by the very low overlapping between the effective irradiation reaching the aqueous solution (> 350 nm) and the AMX absorption spectra (below 350 nm).

Elmolla et al. [20] studied the degradation of different antibiotics, including AMX, by UVA-photolysis (365 nm) and observed that the concentration of AMX decreased in only 2.9% after 300 min. These authors also justify this result indicating that AMX absorbs light only below 300 nm (the maximum absorbance for AMX is found between 230 and 250 nm). Pereira et al. [35] also concluded that AMX is very stable in experiments of photolysis (in this case with a cutoff of 280-300 nm), no significant mineralization being observed for a reaction time of 215 min, even if in their work is shown that AMX has some small absorption of light above 300 nm (Figure 7).

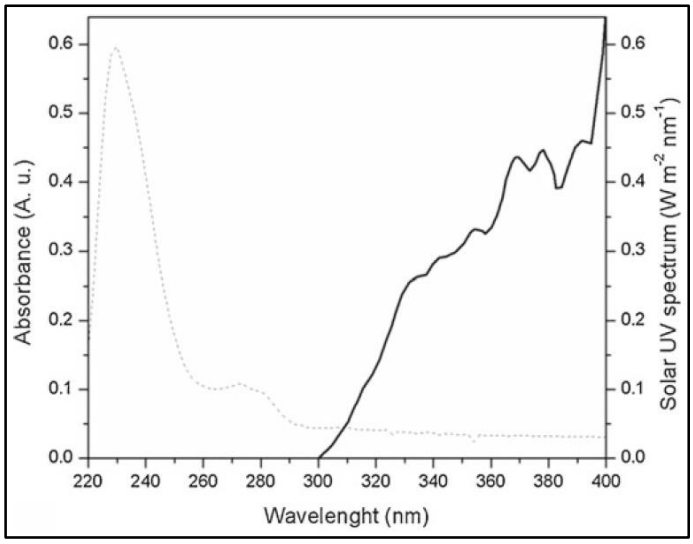



**Figure 7** – Amoxicillin UV absorbance (dashed line) and solar UV spectrum (solid line) adapted from Pereira et al. [35].

Overall, it can be concluded that photolysis by itself, at the employed conditions, is not adequate for treatment of waters containing AMX, but any process implementing ozone (single ozonation, photolytic ozonation and photocatalytic ozonation) are efficient solutions. Having into account installation and operating costs, single ozonation is the most adequate process if the aim is to remove AMX only.

### 4.1.2 Diclofenac (DFC)

DFC was the second organic pollutant studied in this work. By HPLC analysis, it was found that DFC was degraded after 5 min in all experiments performed, i.e. single ozonation, photolytic ozonation, photolysis and photocatalytic ozonation. DFC absorbs at higher wavelengths (up to ca. 325 nm) than AMX and has one maximum at 276 nm (Fig. 8). Therefore, DFC degradation by direct photolysis was not expected, taking into account the effective irradiation reaching the reactor (> 350 nm). However, Pérez-Estrada et al. [44] also observed DFC degradation in solar photolysis, even if its mineralization never occurred. This fact was justified by the tail in DFC absorption spectra, well over 300 nm, allowing absorption of solar photons. In this context, it seems that the light absorption of DFC at wavelengths higher than AMX is in somehow overlapping with the spectra of light reaching the reactor, suggesting that only irradiation with $\lambda$ < 300 nm is cut by the DURAN® glass cooling jacket.

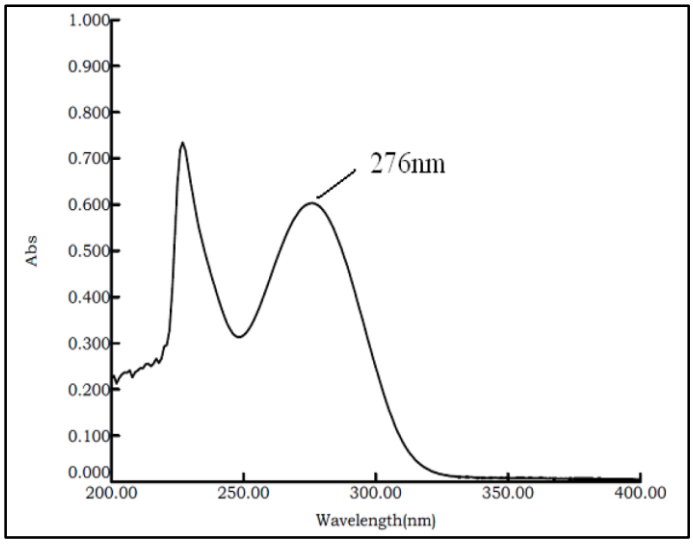



**Figure 8** – Maximum absorption of DFC adapted from Gunji et al. [57].

García-Araya et al. [39] studied the DFC removal in water by using ozone and $TiO_2$ photocatalysis separately. The results showed that DFC was completely eliminated with single ozonation; however, the TOC removal was low, reaching a maximum value of 50%. Relatively to the photocatalytic reaction, DFC was also completely removed, but in this case higher mineralization was observed (ca. 80%). Even so, the resulting intermediate products can be more toxic than the parent species [58]. Aguinaco et al. [38] studied the DFC removal by photocatalytic ozonation and verified a complete degradation of DFC after 6 min of reaction. Since DFC has a nucleophilic site (an amino group), it is expected a fast reaction with an electrophilic agent, such as ozone.

## 4.2 pH, oxalic and oxamic acid evolution

Due to the possibility of by-products formation during the oxidation reactions, it is important to investigate the evolution of the TOC concentration along the experiment, as well as some typical by-products formed in AOPs, such as oxalic and oxamic acids. The aqueous solution pH was also measured before and after each experiment.

### 4.2.1 Amoxicillin (AMX)

The initial and final pH values for samples containing AMX are shown in Table 4.

**Table 4** – Initial and final (after 3 h of treatment) pH for different experiments performed AMX.

| Process | Initial pH | Final pH |
|---|---|---|
| **Ozonation** | 4.0 | 3.5 |
| **Photolysis** | 4.0 | 3.4 |
| **Photolytic ozonation** | 4.2 | 3.3 |
| **Photocatalytic ozonation** | 3.8 | 3.3 |

The slight differences of the initial pH values (4.0 ± 0.2) can be related to small variations in the pH of the distilled water that was used to prepare the initial AMX solutions, or even to some deviations of the pH meter. The initial and final pH values were very similar for all processes tested, but the pH slightly decreased, what might be explained by the formation of by-products or $CO_2$.

An experiment with ultrapure water was realized, in order to verify the effect of ozone, oxygen and UV radiation in pH. A decrease on pH values occurred for both of the experiments ($pH_i$ ($O_3$+UV = 4.392), $pH_f$ ($O_3$+UV = 4.038), $pH_i$ ($O_2$+UV = 4.505), $pH_f$ ($O_2$+UV = 4.007). This might explain the decrease of the pH values for photolysis.

For the experiments with AMX, the concentration of oxalic and oxamic acids over time are shown in Figures 9 and 10, respectively.

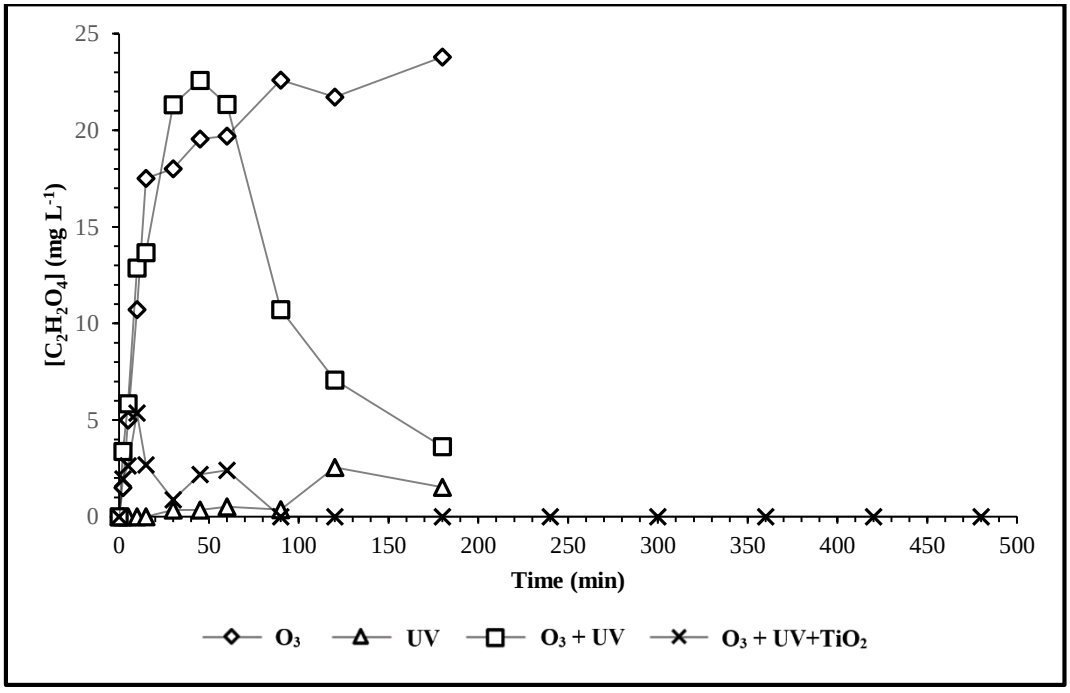



**Figure 9** – Oxalic acid concentration evolution for single ozonation, photolysis, photolytic ozonation and photocatalytic ozonation in experiments performed with AMX.

By the analysis of Figures 9 and 10, the concentration of oxalic and oxamic acids increased during the reaction for single ozonation, although there is a higher increase in the initial 30 min. These figures also suggests that the concentration of these by-products tends to constant values after 3 h of reaction, situated between 20 and 25 mg $L^{-1}$ for oxalic acid and near 3 to 4 mg $L^{-1}$ for oxamic acid. These results indicate that the degradation of these by-products is difficult by using single ozonation. This might be explained by the selective attack of ozone at low pH, where the aromatic compounds are primarily attacked and low weight carboxylic acids are of difficult degradation by single ozonation.

Regarding the photolysis experiment, a very small oxalic acid concentration is detected in 45 min, a slight increase is only observed after 90 min and no significant changes were then detected (Figure 9). For oxamic acid, the concentration slightly increases after 45 min but then remains constant over time – 3 h (Figure 10). However, when ozone was added (photolytic ozonation), the concentration of oxalic acid increased faster, up to ca. 22.5 mg $L^{-1}$ in the first hour, and then decreased, to a value close to 3.6 mg $L^{-1}$ in 3 h. Oxamic acid seems more refractory than oxalic acid, since a constant accumulation of oxamic acid (up to 6 mg $L^{-1}$) is observed in photolytic ozonation.

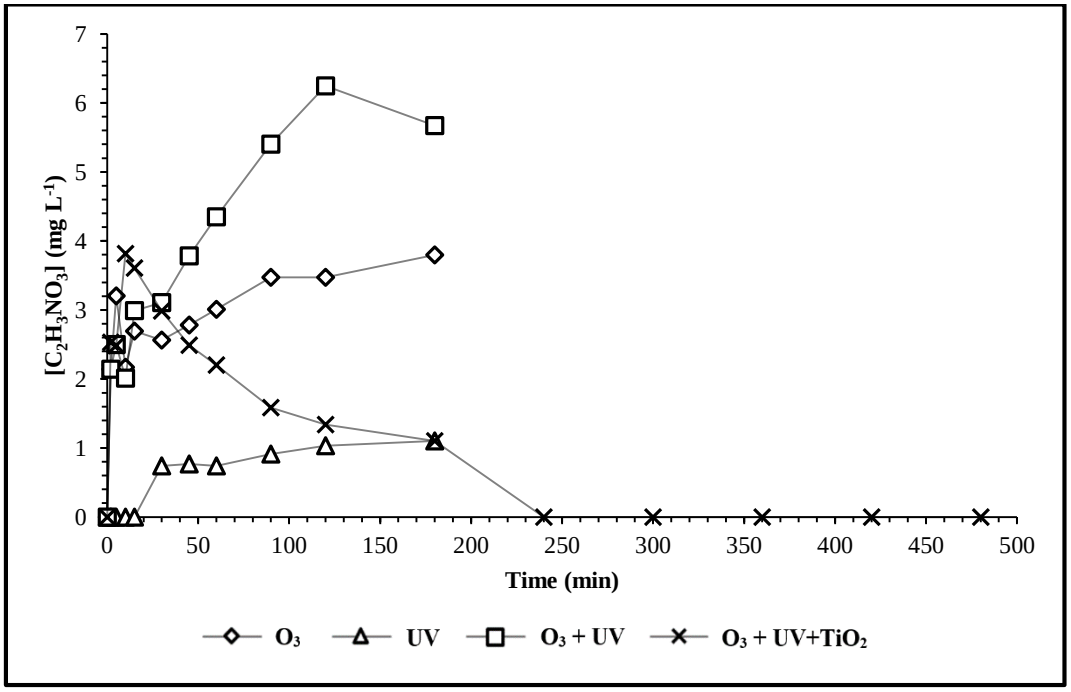



**Figure 10** – Oxamic acid concentration evolution for single ozonation, photolysis, photolytic ozonation and photocatalytic ozonation in experiments performed with AMX.

The best results were obtained by photocatalytic ozonation, where the oxalic acid concentration increases in the first 10 min, but immediately starts to decrease until there is no more oxalic acid in solution (only 90 min are needed). Similar results were observed for oxamic acid, i.e. a rapid increase in acid concentration during the first 10 min (reaching a maxima ca. 3.8 mg $L^{-1}$), the concentration then decreasing until complete degradation of the acid in 240 min. These results suggest that both acids are attacked so fast in photocatalytic ozonation that their concentrations does not increase as high as in the absence of a photocatalyst (i.e. photolytic ozonation), where accumulation of these acids occurs. As explained in Section 1.4., $HO^{\bullet}$ is the main oxidizing radical in photocatalytic ozonation (Eqs 14 – 24), non-selectively oxidizing the organic pollutants and respective intermediate compounds.

### 4.2.2 Diclofenac (DFC)

The initial and final pH values for each experiment performed with DFC are shown in Table 5. A decrease of pH was observed in all experiments, as also verified for AMX.

**Table 5** – Initial and final (after 3 h of treatment) pH for different experiments performed with DFC.

| Reaction | Initial pH | Final pH |
|---|---|---|
| **Single ozonation** | 4.5 | 3.4 |
| **Photolysis** | 4.4 | 3.0 |
| **Photolytic ozonation** | 5.1 | 3.3 |
| **Photocatalytic ozonation** | 4.6 | 4.1 |

The oxalic and oxamic acid concentrations versus time are shown in Figures 11 and 12, respectively, for experiments performed with DFC. Figure 11 shows that the concentration of oxalic acid reaches a similar maximum for ozonation and photolytic ozonation in 45 min. However, the oxalic acid concentration remains constant in single ozonation, while its concentration decreases in photolytic ozonation (this decrease slow down after 90 min). In photolysis, oxalic acid is not formed throughout the reaction. In photocatalytic ozonation this acid is rapidly formed and degraded in the first 15 min.

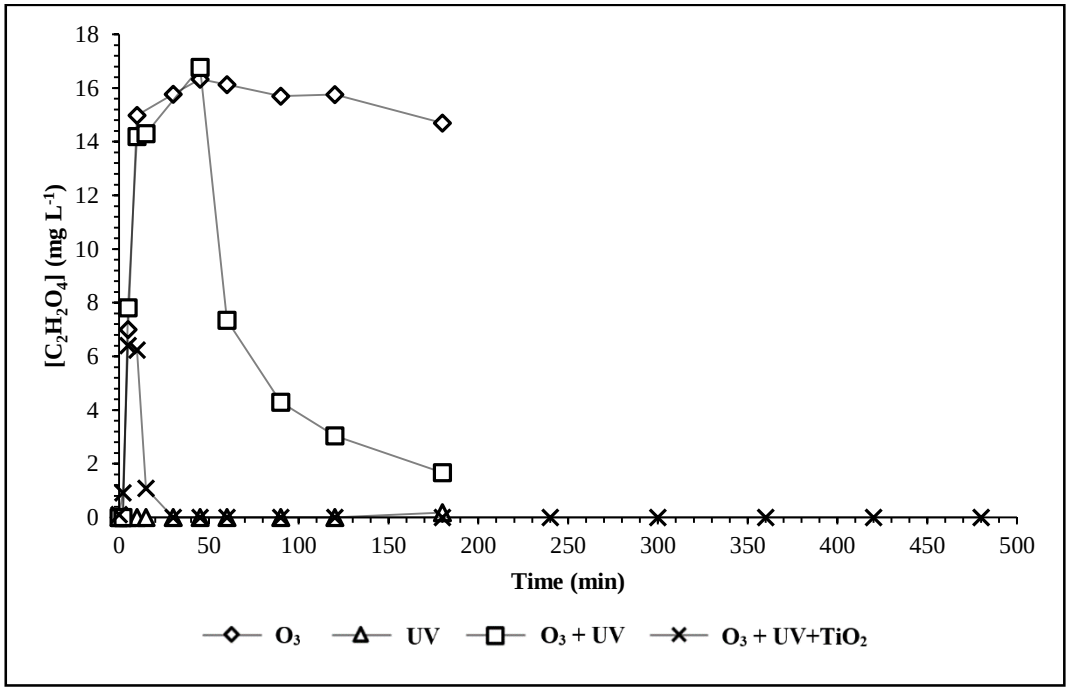



**Figure 11** – Oxalic acid concentration evolution for single ozonation, photolysis, photolytic ozonation and photocatalytic ozonation in experiments performed with DFC.

In experiments performed with DFC the oxamic acid concentration follows a similar behavior to that observed for AMX. An increase of the concentration until about 120 min is observed in single ozonation, only a low decrease being observed after 120 min.

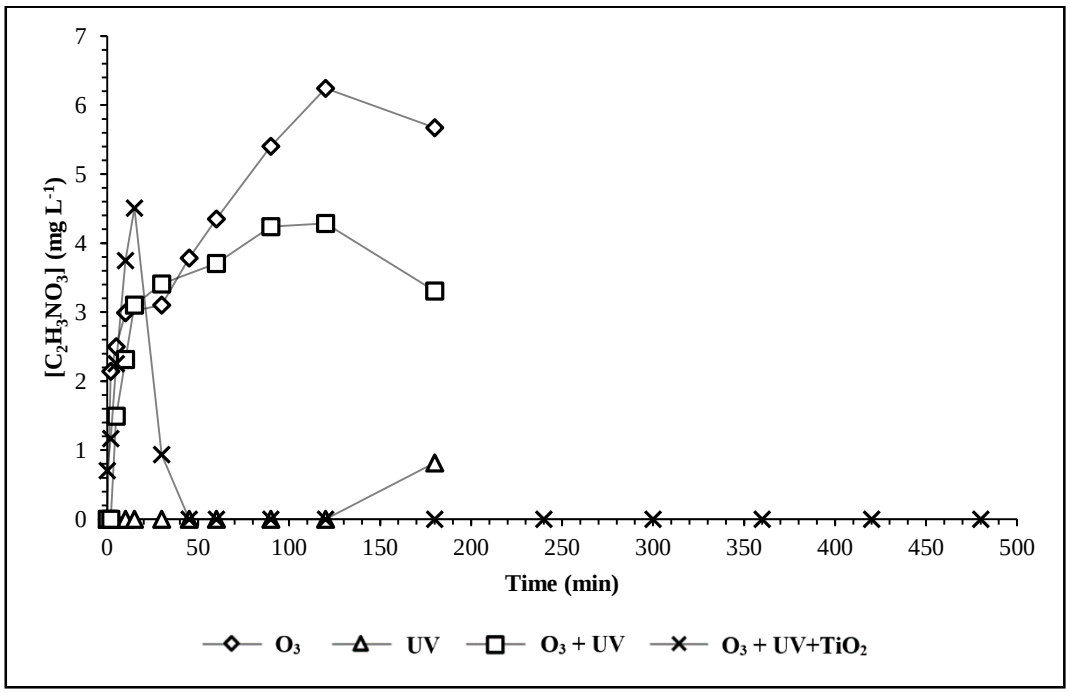



**Figure 12**– Oxamic acid concentration evolution for single ozonation, photolysis, photolytic ozonation and photocatalytic ozonation in experiments performed with DFC.

The same is true for the photolytic ozonation reaction, but the maximum concentration achieved is lower. The formation of oxamic acid in photolysis occurs only after 120 min. Finally, a significant increase in oxamic acid concentration was possible with photocatalytic ozonation in the very first minutes of reaction, the concentration decreasing after 15 min until complete degradation of this acid in 45 min.

## 4.3 Total organic carbon (TOC) evolution

TOC is a very important parameter to consider since it allows to identify the amount of organic matter in solution and verifies the degree of mineralization of the compounds for a given treatment. In this area of study, it becomes even more important since although from HPLC analysis the initial contaminants were completely degraded in some experiments, this does not guarantee that all by-products were also degraded. The normalized TOC concentration ($TOC/TOC_0$) *vs.* time for all experiments performed with AMX and DFC is shown in Figures 13 and 14, respectively.

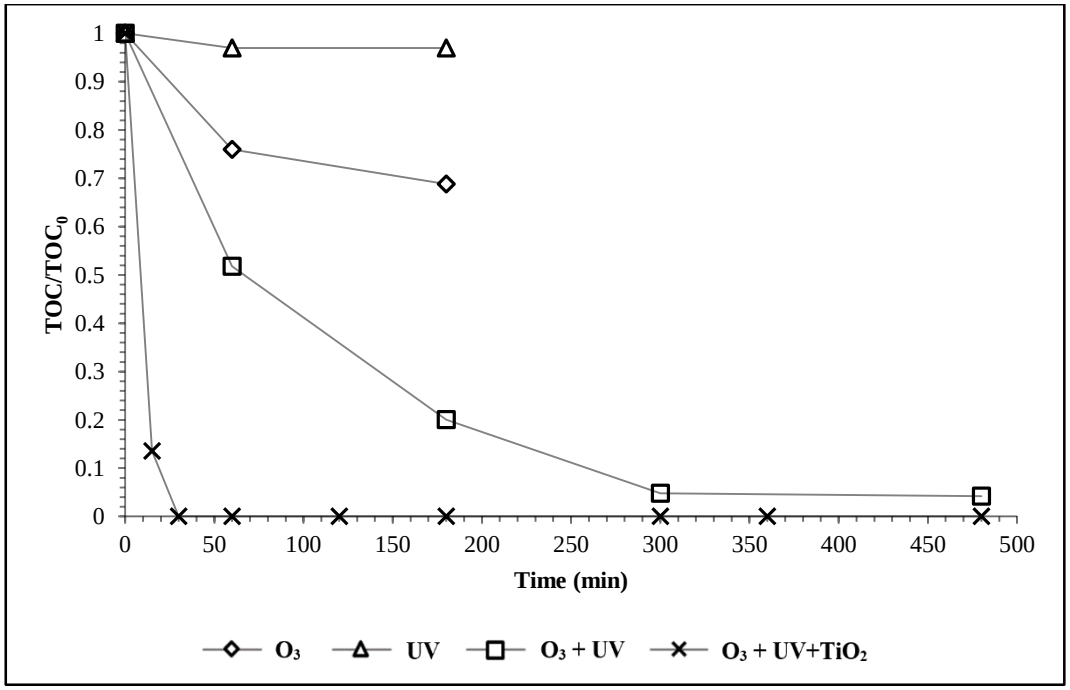



**Figure 13** – TOC removal for AMX during single ozonation, photolysis, photolytic ozonation and photocatalytic ozonation.

The TOC variation is identical for the two model pollutants comparing the same AOP. In photolysis, a low decrease on the TOC values is observed in experiments with both pollutants, never reaching more than 6% of TOC removal. The mineralization is higher for single ozonation, but never exceeding 41%. The TOC decrease is more significant in photolytic ozonation, reaching higher TOC removals with both pollutants in 300 min, as high as 96%. Regarding photocatalytic ozonation, it is possible to achieve TOC removals of 100%, for AMX and DFC in 30 and 120 min, respectively. Performing experiments with DFC, Aguinaco et al. [38] also studied the TOC removal in photolysis, photocatalytic oxidation and photocatalytic ozonation and concluded that the last one has the highest TOC removal, approximately 90%.

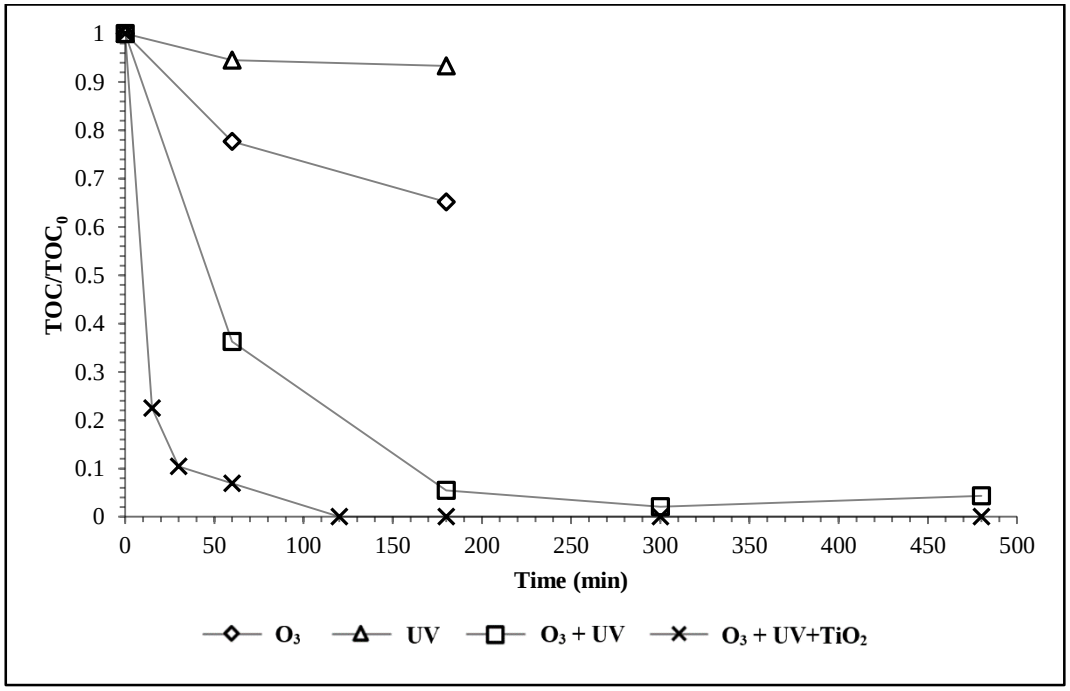



**Figure 14** – TOC removal for DFC during single ozonation, photolysis, photolytic ozonation and photocatalytic ozonation.

Therefore, it was possible to verify a synergistic effect when the photocatalytic and ozonation treatments were carried out simultaneously, as also verified by Wang et al. [19] when studied the degradation of formic acid. It is possible to consider a main mechanism in photocatalytic ozonation, namely that dissolved ozone reacts easier with electrons produced on the $TiO_2$ surface (Eqs 1 and 14), avoiding the recombination of the positive holes with the electrons. This reaction will lead to the production of a large amount of oxidizing radicals, accelerating the degradation process. Other reactions can

simultaneously occur, but more detailed studies are needed to understand the overall mechanism taking place.

## 4.4 Experiments with a synthetic wastewater

The impact of photolytic ozonation on TOC removal was also studied using AMX in a synthetic wastewater prepared in the laboratory (as explained in Section 3). As future work it is suggested to perform this type of experiments for the most efficient AOP identified in the present work (i.e. photocatalytic ozonation), considering both AMX and DFC pollutants. This type of experiments better simulates real effluents and they are important because the TOC removal can be lower when the aqueous solution matrix is different.

Two experiments were performed, one with the synthetic wastewater only and the other with AMX (0.1 mM) added to the synthetic wastewater. The composition of the synthetic wastewater was selected accordingly to the requirements for discharges of effluent from urban WWTPs, subject to the provisions of Articles 5 and 6 from Decreto-Lei n. º 152/97, June 19. BOD and COD are the legislated parameters and, thus, they were monitored before and after treatment (Table 6). These parameters were measured in the laboratory, and the initial ones confirmed by a SME (Adventech, São João da Madeira). The TOC was also measured in these experiments; an initial value of 39.3 mg $L^{-1}$ was determined for the synthetic effluent.

**Table 6** – BOD and COD values determined from the synthetic wastewater before and after photolytic ozonation.

| | $BOD_{initial}$ (mg $L^{-1}$) | $BOD_{after\ treatment}$ (mg $L^{-1}$) | $COD_{initial}$ (mg $L^{-1}$) | $COD_{after\ treatment}$ (mg $L^{-1}$) |
|---|---|---|---|---|
| **Laboratory** | 26 | 4 | 118 | 66 |
| **Adventech** | 30 | -- | 115 | -- |

By the results in Table 6, it was possible to verify a decrease in both BOD and COD values after treatment. Near 35% and 44% of TOC removals were obtained in experiments with and without AMX, respectively, after 3 h of treatment. Despina et al. [31] investigated the UV-A/$TiO_2$ decomposition of AMX and used two different matrices, ultrapure water and secondary treated effluent, and concluded that the degradation in treated effluent was partly impeded compared to pure water due to the inherent presence

of organic and inorganic compounds that may compete for hydroxyl radicals. The rate of conversion depends on the substrate to oxidizing species concentration ratio. Robab et al. [3] studied the mineralization of AMX (20 mg $L^{-1}$) in a real water using $Sn/TiO_2$ nanoparticles as photocatalysts and concluded that in the presence of some anions, such as sulfate, carbonate and bicarbonate, the mineralization decreases. Ions can even block the active sites on catalyst surface, thus deactivating the catalyst towards AMX degradation. A series of reactions showing the ability of anions to act as $OH^{\bullet}$ scavengers are presented in Eqs. 26–28 [3]:

$$SO_4^{2-} + OH^{\bullet} \rightarrow SO_4^{\bullet -} + OH^- \quad (26)$$

$$HCO_3^- + OH^{\bullet} \rightarrow CO_3^{\bullet -} + H_2O \quad (27)$$

$$CO_3^{2-} + OH^{\bullet} \rightarrow CO_3^{\bullet -} + OH^- \quad (28)$$

This may explain why the TOC removal is lower when AMX is dissolved in a synthetic wastewater when comparing with the AMX dissolved in ultrapure water. In order to verify if the presence of microorganisms in the water has a significant impact in TOC value, a suspension of *E. coli* in ultrapure water at the same density used in the assays ($10^3$ CFU $mL^{-1}$) was prepared and analyzed. The initial TOC was very low, equal to the ultrapure water value, approximately 2 mg $L^{-1}$, so it is possible to conclude that no significant increase in TOC value was caused by the addition of the test organism to the synthetic water.

## 4.5 Ions concentration evolution

The concentration of different ions was analyzed in the different experiments. The determination of these ions is important in order to understand what is happening to the initial compound after being submitted to treatment and, in particular, to estimate if non-identified compounds were formed. The concentrations of nitrates, nitrites, ammonium, sulfates and chlorides are presented in Tables 7 and 8, for the experiments with AMX and DFC, respectively.

**Table 7** – Nitrates, nitrites, ammonium and sulfates concentration for different experiments performed with AMX.

| | Time (min) / Process | 0 | 60 | 180 | 480 |
|---|---|---|---|---|---|
| **Nitrates (mM)** | $O_3$ | < DL | < DL | 0.008 | -- |
| | UV | < DL | < DL | 0.015 | -- |
| | $O_3$+UV | < DL | 0.014 | 0.033 | -- |
| | $O_3$+UV+$TiO_2$ | < DL | 0.094 | -- | 0.167 |
| **Nitrites (mM)** | $O_3$ | < DL | < DL | < DL | -- |
| | UV | < DL | < DL | < DL | -- |
| | $O_3$+UV | < DL | < DL | < DL | -- |
| | $O_3$+UV+$TiO_2$ | < DL | < DL | -- | < DL |
| **Ammonium (mM)** | $O_3$ | < DL | < DL | 0.063 | -- |
| | UV | < DL | < DL | < DL | -- |
| | $O_3$+UV | < DL | 0.063 | 0.079 | -- |
| | $O_3$+UV+$TiO_2$ | < DL | 0.097 | -- | 0.077 |
| **Sulfates (mM)** | $O_3$ | < DL | < DL | < DL | -- |
| | UV | < DL | < DL | < DL | -- |
| | $O_3$+UV | < DL | < DL | 0.022 | -- |
| | $O_3$+UV+$TiO_2$ | < DL | 0.088 | -- | 0.095 |

$DL_{Nitrates}$ – 0.005 mM; $DL_{Nitrites}$– 0.008 mM; $DL_{Amonnium}$ – 0.005 mM; $DL_{Sulfates}$– 0.009 mM;

**Table 8** – Nitrates, nitrites, ammonium and chlorides for different experiments performed with DFC.

| | Time (min) / Reaction | 0 | 60 | 180 | 480 |
|---|---|---|---|---|---|
| **Nitrates (mM)** | $O_3$ | < DL | < DL | 0.005 | -- |
| | UV | < DL | < DL | < DL | -- |
| | $O_3$+UV | < DL | < DL | 0.038 | -- |
| | $O_3$+UV+$TiO_2$ | < DL | 0.055 | -- | 0.054 |
| **Nitrites (mM)** | $O_3$ | < DL | < DL | < DL | -- |
| | UV | < DL | < DL | < DL | -- |
| | $O_3$+UV | < DL | < DL | < DL | -- |
| | $O_3$+UV+$TiO_2$ | < DL | < DL | -- | <DL |
| **Ammonium (mM)** | $O_3$ | < DL | 0.030 | 0.006 | -- |
| | UV | < DL | < DL | < DL | -- |
| | $O_3$+UV | < DL | 0.032 | 0.009 | -- |
| | $O_3$+UV+$TiO_2$ | < DL | 0.058 | -- | 0.046 |
| **Chlorides (mM)** | $O_3$ | < DL | 0.168 | 0.169 | -- |
| | UV | < DL | 0.190 | 0.186 | -- |
| | $O_3$+UV | < DL | 0.179 | 0.175 | -- |
| | $O_3$+UV+$TiO_2$ | < DL | 0.201 | -- | 0.209 |

$DL_{Nitrates}$ – 0.005 mM; $DL_{Nitrites}$– 0.008; $DL_{Amonnium}$ – 0.005 mM; $DL_{Chlorides}$ – 0.03 mM;

A balance to the ions concentrations in solution was also made. The balance to nitrogen and sulfur in samples containing AMX for different processes is shown in Tables 9 and 10.

**Table 9** – Nitrogen (N) in AMX, oxamic acid, ammonium, nitrates, nitrites and non-identified compounds ($N_{NI}$) for experiments performed with AMX. The theoretical value of N formed from total AMX conversion is shown for comparison ($N_{AMX\ Possible}$).

| **Process** | **$O_3$** | **UV** | **$O_3$+UV** | **$O_3$+UV+$TiO_2$** | **$O_3$** | **UV** | **$O_3$+UV** | **$O_3$+UV+$TiO_2$** |
|---|---|---|---|---|---|---|---|---|
| **Time (min)** | | | **60** | | | **180** | | **480** |
| **$N_{AMX\ Possible}$ (mM)** | | | 0.3 | | | | 0.3 | |
| **$N_{AMX}$ (mM)** | -- | 0.3 | -- | -- | -- | 0.285 | -- | -- |
| **$N_{oxamic\ acid}$ (mM)** | 0.034 | < DL | 0.049 | < DL | 0.042 | < DL | 0.063 | < DL |
| **$N_{NH_4^+}$ (mM)** | < DL | < DL | 0.063 | 0.097 | 0.063 | < DL | 0.079 | 0.077 |
| **$N_{NO_3^-}$ (mM)** | < DL | < DL | 0.014 | 0.094 | 0.008 | 0.015 | 0.033 | 0.167 |
| **$N_{NO_2^-}$ (mM)** | < DL | < DL | < DL | < DL | < DL | < DL | < DL | < DL |
| **Sum (mM)** | 0.034 | < DL | 0.127 | 0.191 | 0.113 | 0.015 | 0.175 | 0.245 |
| **$N_{NI}$ (mM)** | 0.266 | -- | 0.173 | 0.109 | 0.187 | -- | 0.125 | 0.055 |

$DL_{Nitrates}$ – 0.005 mM; $DL_{Nitrites}$– 0.008; $DL_{Amonnium}$ – 0.005 mM; $DL_{oxamic\ acid}$ – 0.03 mM;

By the results shown in Tables 9 and 10, it was possible to verify different variations in the species containing nitrogen (resulting in nitrates, oxamic acid and ammonium) and sulfur (resulting in sulfates) in experiments with AMX. The identification of all nitrogen-containing and sulfur-containing compounds was not possible for most of the reactions studied, as shown by the $N_{NI}$ and $S_{NI}$ parameters.

**Table 10** – Sulfur (S) in AMX, sulfates and non-identified compounds ($S_{NI}$) for experiments performed with AMX. The theoretical value of S for total AMX conversion is shown for comparison ($S_{AMX\ Possible}$)

| **Process** | **$O_3$** | **UV** | **$O_3$+UV** | **$O_3$+UV+$TiO_2$** | **$O_3$** | **UV** | **$O_3$+UV** | **$O_3$+UV+$TiO_2$** |
|---|---|---|---|---|---|---|---|---|
| **Time (min)** | | | 60 | | | 180 | | 480 |
| **$S_{AMX\ Possible}$ (mM)** | | | 0.1 | | | | 0.1 | |
| **$S_{AMX}$ (mM)** | -- | 0.1 | -- | -- | -- | 0.1 | -- | -- |
| **$S_{SO_4^{2-}}$ (mM)** | < DL | < DL | < DL | 0.088 | < DL | < DL | 0.022 | 0.095 |
| **$S_{NI}$ (mM)** | 0.1 | -- | 0.1 | 0.012 | 0.1 | -- | 0.078 | 0.005 |

$DL_{Sulfates}$– 0.009 mM;

In photolysis, AMX is not degraded and, as expected, just 5% of nitrogen appears as nitrates. No sulfur-containing by products were identified. For single ozonation, 14% of nitrogen appears as oxamic acid, 21% as ammonium, 2.7% as nitrates and 62% as non-

identified compounds, after 180 min of reaction. In photolytic ozonation, 21% of nitrogen was identified as oxamic acid, 26% as ammonium, 11% as nitrates and ca. 41% of nitrogen species were not identified after 180 min. Relatively to sulfur, 22% was identified as sulfates for photolytic ozonation after 180 min.

For photocatalytic ozonation, approximately 25% of nitrogen was identified as ammonium and 56% as nitrates after 8 hours of treatment. Nitrites were not observed and oxamic acid was completely degraded during the reaction. It is also possible that 18% of nitrogen was converted to $N_2$. In addition, 95% of AMX conversion resulted into sulfates.

The results obtained agree with the mineralization obtained in each process.

The balance to nitrogen and chlorine in experiments with DFC for different reactions is presented in Tables 11 and 12.

**Table 11** – Nitrogen (N) in DFC, oxamic acid, ammonium, nitrates, nitrites and non-identified compounds ($N_{NI}$) for experiments performed with DFC. The theoretical value of N formed from total DFC conversion is shown for comparison ($N_{DFC\ Possible}$)

| **Process** | **$O_3$** | **UV** | **$O_3$+UV** | **$O_3$+UV+$TiO_2$** | **$O_3$** | **UV** | **$O_3$+UV** | **$O_3$+UV+$TiO_2$** |
|---|---|---|---|---|---|---|---|---|
| **Time (min)** | | | 60 | | | 180 | | 480 |
| **$N_{DFC\ Possible}$ (mM)** | | | 0.1 | | | | 0.1 | |
| **$N_{DFC}$ (mM)** | -- | -- | -- | -- | -- | -- | -- | -- |
| **$N_{oxamic\ acid}$ (mM)** | 0.049 | < DL | 0.042 | < DL | 0.064 | < DL | 0.037 | -- |
| **$N_{NH_4^+}$ (mM)** | 0.033 | < DL | 0.032 | 0.056 | < DL | < DL | 0.009 | 0.048 |
| **$N_{NO_3^-}$ (mM)** | < DL | < DL | < DL | 0.055 | 0.005 | < DL | 0.038 | 0.054 |
| **$N_{NO_2^-}$ (mM)** | < DL | < DL | < DL | < DL | < DL | < DL | < DL | < DL |
| **Sum (mM)** | 0.082 | -- | 0.074 | 0.111 | 0.069 | -- | 0.084 | 0.103 |
| **$N_{NI}$ (mM)** | 0.018 | 0.1 | 0.026 | -- | 0.031 | 0.1 | 0.016 | -- |

$DL_{Nitrates}$ – 0.005 mM; $DL_{Nitrites}$ – 0.008; $DL_{Amonnium}$ – 0.005 mM; $DL_{oxamic\ acid}$ – 0.03 mM;

Table 11 shows that nitrogen conversion was not identified after 180 min of photolysis. 93% of chlorine was identified as chlorides. These results might explain why in photolysis was possible to degrade DFC but with low TOC removal. For single ozonation, after 180 min, the distribution of nitrogen was 64% as oxamic acid, 5% as nitrates and 31% of non-identified compounds. Relatively to chlorine, 84% was identified as chlorides. In photolytic ozonation, nitrogen was identified 37% as oxamic acid, 9% as ammonium, 38% as nitrates and 16% of non-identified compounds. 87% of the initial chlorine originated chlorides. Finally, for photocatalytic ozonation, the final distribution of nitrogen was 48% as ammonium and 54% as nitrates. All the chlorine was converted to

chlorides. These results are once again in agreement with the TOC removals verified for the different reactions.

**Table 12** – Chlorine in DFC, chlorides and non-identified compounds ($CL_{NI}$) for experiments performed with DFC. The theoretical value of Cl for total DFC conversion is shown for comparison ($Cl_{AMX\ Possible}$)

| **Process** | **$O_3$** | **UV** | **$O_3$+UV** | **$O_3$+UV+$TiO_2$** | **$O_3$** | **UV** | **$O_3$+UV** | **$O_3$+UV+$TiO_2$** |
|---|---|---|---|---|---|---|---|---|
| **Time (min)** | | | 60 | | | 180 | | 480 |
| **$Cl_{DFC\ Possible}$ (mM)** | | | 0.2 | | | | 0.2 | |
| **$Cl_{DFC}$ (mM)** | -- | -- | -- | -- | -- | -- | -- | -- |
| **$C_{Cl^-}$ (mM)** | 0.168 | 0.190 | 0.179 | 0.201 | 0.169 | 0.186 | 0.175 | 0.209 |
| **$Cl_{NI}$ (mM)** | 0.032 | 0.010 | 0.021 | -- | 0.031 | 0.014 | 0.025 | -- |

$DL_{Chlorides}$ – 0.03 mM

In some cases ion concentrations higher than the theoretical values were obtained for N and Cl formed from total DFC conversion, which might be associated with analytical errors.

## 4.6 Effectiveness of photolytic ozonation on bacterial inactivation

Subsequently, a test to verify the effect of advanced oxidation processes in the inactivation of microorganisms present in the water was carried out. The legislation for wastewater reuse requires monitoring of biological parameters, therefore it is important to reduce the microbial density of the effluent. If photolytic ozonation permits, at the same time, the reduction or even elimination of all microbial organisms and the degradation of organic compounds, it becomes a very interesting process in the treatment of wastewater effluents. The results obtained after photolytic ozonation using the membrane filter method to count the viable organisms remaining in solution, are presented in Table 13.

**Table 13** – Microbial density of *E. coli* before and after photolytic ozonation for different times and dilutions. [a]

| | | | LA (CFU $mL^{-1}$) | | m-FC (CFU $mL^{-1}$) | |
|---|---|---|---|---|---|---|
| | Dilutions | Volume (mL) | 1 | 2 | 1 | 2 |
| **Initial time without treatment** | $10^{-1}$ | 1 | $1.02 \times 10^3$ | $1.20 \times 10^3$ | $4.70 \times 10^2$ | $6.10 \times 10^2$ |
| | $10^{-2}$ | 1 | $1.60 \times 10^3$ | $1.40 \times 10^3$ | $3.00 \times 10^2$ | $5.00 \times 10^3$ |
| **After 3 hours without treatment** | $10^{-2}$ | 1 | $1.70 \times 10^3$ | $4.70 \times 10^3$ | $1.40 \times 10^3$ | $1.00 \times 10^3$ |
| | $10^{-3}$ | 1 | $3.00 \times 10^3$ | $3.00 \times 10^3$ | $1.00 \times 10^3$ | 0 |
| **After 3 hours with treatment** | $10^{-1}$ | 1 | 0 | 0 | 0 | 0 |
| | $10^{-2}$ | 1 | 0 | 0 | 0 | 0 |
| | $10^{-3}$ | 1 | 0 | 0 | 0 | 0 |
| **After 120 hours without treatment** | $10^{-2}$ | 1 | N.C. | N.C. | N.C. | N.C. |
| | $10^{-3}$ | 1 | N.C. | N.C. | N.C. | N.C. |
| | $10^{-4}$ | 1 | N.C. | N.C. | N.C. | N.C. |
| **After 120 hours with treatment** | - | 1, 10, 100 | 0 | 0 | 0 | 0 |
| **After 180 hours without treatment** | $10^{-4}$ | 1 | N.C. | N.C. | N.C. | N.C. |
| | $10^{-5}$ | 1 | N.C. | N.C. | N.C. | N.C. |
| | $10^{-6}$ | 1 | $2.7 \times 10^7$ | $2.8 \times 10^7$ | $1.0 \times 10^7$ | $2.0 \times 10^7$ |
| **After 180 hours with treatment** | - | 1, 10, 100 | 0 | 0 | 0 | 0 |

[a] N.C. – Not countable.

By the results presented in Table 13 it is possible to verify that the photolytic ozonation inactivated *E. coli*. In order to confirm if the treatment just inhibited the growth for a short period of time, filtrations were made after 120 and 180 hours after treatment and it was concluded that *E. coli* was eliminated from water. The UVA radiation can lead to cellular membrane damage, since during the process occurs the production of reactive oxygen species, such as $O_2^{\bullet}$ and $OH^{\bullet}$ generated via excitation of dissolved oxygen in water [59]. $H_2O_2$ and $HO^{\bullet}$ can be also formed by the ozone reaction with UV-light. The reactive oxygen species formed can damage the outer membrane of *E. coli* cells, and by this way, facilitating the access of radical species to the cytoplasmic membrane. Posteriorly a lipid peroxidation occurs leading to a disorder in the structure of the cytoplasmic membrane, losing permeability. This lost will promote the entrance of reactive species leading to the attack of intracellular components, for example, proteins and nucleic acids. It is also possible to occur the inhibition of respiration by the depletion of coenzyme A [10, 60].

Khaengraeng et al. [61] demonstrated that a substantial proportion of *E. coli* cells subjected to either UVA or simulated sunlight become sublethally damage and can no

longer form colonies in air on an agar-based medium. Ozone is a very powerful oxidant by-itself, so despite its highly efficient inactivation of microorganisms present in the water, ozonation can also produce disinfection by-products. The temperature is other factor that can have impact in disinfection. The increase of temperature generally provokes an increase in the disinfection power. The composition of water can affect the efficiency of UV disinfection, principally, the optical properties of water. The presence of fecal material, and dissolved solid materials protect the microorganisms against radiation effect [1].

Sousa et al. [60] studied the photoinactivation of various antibiotic resistant strains of *E. coli* using a paint coat, and verified viability losses above 98.7% for cells contacting the surface of the photocatalytic paint coat and 99.5% for cells in solution, after 40 min of 10 W $m^{-2}$ UV-A exposure. Robertson et al. [10] studied the effectiveness of $TiO_2$ photocatalysis and UV-A photolysis for the destruction of *E. coli*, and concluded that $TiO_2$ photocatalysis lead to a greater disinfection after 120 min. Although UV-A have a big disinfection potential, small colony variants of target pathogenic organisms can be produced increasing the risk of producing infection with a pathogen that is more difficult to treat. In conclusion, when the UV-A light is used alone, the microorganism may reactivate, which does not happen in the $TiO_2$ photocatalytic system.

In this work the use of ozone might be the principal factor in the inactivation and elimination of *E. coli*. Finally, it was possible to conclude that photolytic ozonation is an efficient process for water disinfection.

## 4.7 Growth inhibition assay for samples containing AMX and DFC

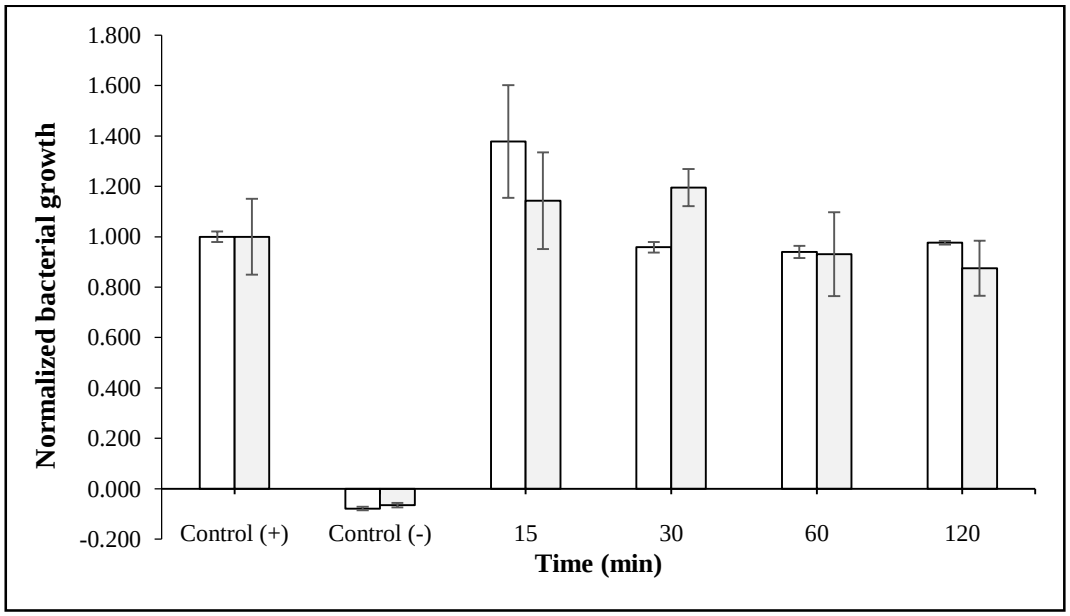
The induced biological response in different microorganisms caused by a chemical substance is very diverse and relies on their sensitivity to that compound. So, in order to verify the toxicity of a chemical substance is important to combine toxicity bioassays with chemical analysis of target compounds. The commonly-used bioassay methods are nowadays replaced by microbial bioassays and biosensors. These tests can assess toxicity by their effects on living cells or organelles directly and it is a faster method to achieve results. They are better in an ethical point of view, since no higher organisms are used [29, 62].

A growth inhibition assay was performed for samples containing AMX and DFC. The process chosen to be tested was photocatalytic ozonation, since it was the process with better results, where a complete mineralization after 15 minutes of reaction was verified.

Specific growth rate (μ, $h^{-1}$) was calculated, for each well, by adjusting the experimental data of the exponential growth phase to a first-order kinetic model and the biomass yield (Y) by the difference between maximum OD achieved and initial OD. Then the values were normalized by the positive control. *E. coli* (DSM 1103) and *S. aureus* (DSM 1104) were used as test strains.

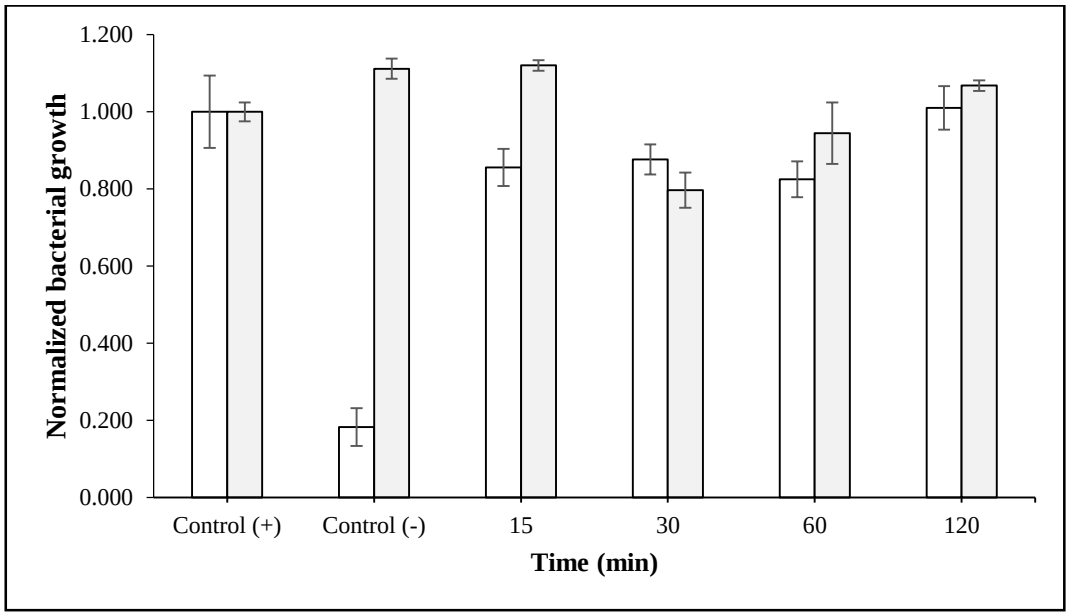
The normalized bacterial growth rate and biomass yield values of *S. aureus* and *E. coli*, for samples containing AMX and DFC are presented in Figure 15.

**(a)**

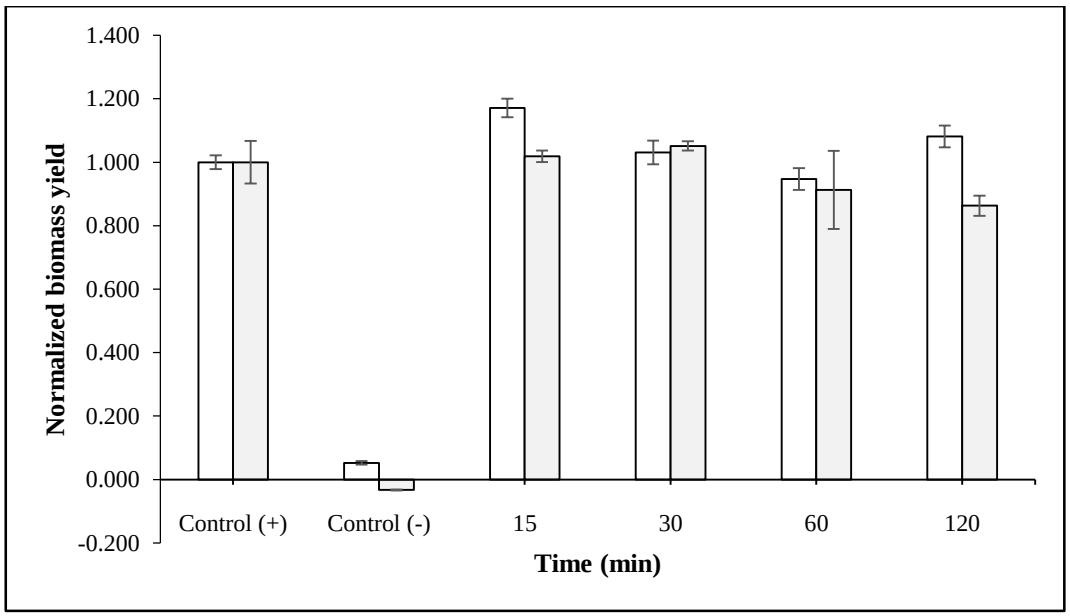



**(Continued in next page)**

**(b)**




**(c)**




**(Continued in next page)**

**(d)**

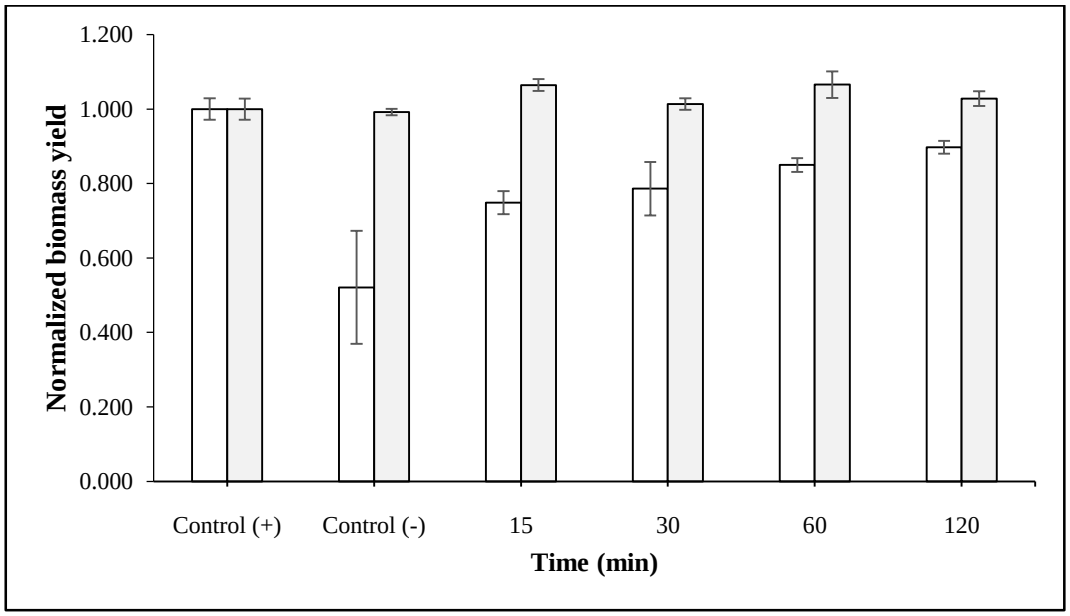



**Figure 15** - Staphylococcus aureus (white columns) and Escherichia coli (gray columns) normalized bacterial growth rate and normalized biomass yield for samples containing AMX (**a)** and **(c)** and DFC (**b)** and **(d)**, for different reaction times compared to the positive control (Control (+)).

The main objective of this test was to verify if AMX and DFC have toxic properties and if during the photocatalytic ozonation compounds even more toxic than the original organic compounds are formed. If the compounds do not lose their antimicrobial and toxic properties, they will be released into water sources and will contribute to the development of multiresistant microorganisms to antibiotics. If antibiotics and antibiotic resistant bacteria are not eliminated after wastewater treatment, spreading of antibiotic resistance among bacteria may occur. Selection of resistant bacteria can occur at contaminant concentrations lower than the minimal inhibitory concentrations. Due to this problem, it is very important that AMX and DFC are eliminated from the effluent preventing the antimicrobial drug resistance.

By the results for samples containing AMX showed in Figure 15 (a) it was possible to verify that the initial solution (negative control) inhibited the growth of both strains, which was expected since AMX is an antibiotic, and it was at a concentration (36.5 mg $L^{-1}$) above the minimal inhibitory concentration for the test organisms (4.0 and 0.25 $mg_{AMX}$ $L^{-1}$, for *E. coli* and *S. aureus*, respectively) [38]. As expected, bacterial growth occurred in the positive control (absence of AMX). The results show that growth occurred in all the samples collected over the treatment (15, 30, 60 and 120 min). Thus, it is

possible to conclude that 15 min of reaction was sufficient to inactivate the AMX antimicrobial properties, allowing *S. aureus* and *E. coli* to growth. The formation of more toxic compounds was also not verified, since the by-products of AMX photocatalytic ozonation did not inhibit the bacterial growth. It is possible to conclude that photocatalytic ozonation is a good process to treat water contaminated with AMX, allowing its complete mineralization without the formation of toxic compounds to microbiological strains.

Relatively to DFC, by the results shown in Figure 15 (b), it was possible to verify bacterial growth in the negative control, but far more accentuated for *E. coli*, which indicates a higher resistance for this strain compared with *S. aureus*, for which some inhibition was verified. These results can be explained by the fact of DFC not being naturally an antimicrobial compound. According to Noton et al. [63] DFC has bactericidal effect for higher concentrations (between 50 to 200 $\mu g_{DFC}$ $mL^{-1}$ for *S.aureus* and 50 to $\geq$ 800 $\mu g_{DFC}$ $mL^{-1}$ for *E. coli)* than those used in this experiment (36.5 mg $L^{-1}$). Dastidar et al. [64] also studied the anti-bacterial action of DFC and concluded that most strains of Gram-positive and Gram-negative bacteria were inhibited by 50-100 mg $L^{-1}$ and the anti-bacterial action was found to be due to inhibition of DNA synthesis.

So, it can be concluded that the growth inhibition assay using these two strains may not be the best assay for this type of organic compound.

But otherwise, this assay allowed to verify that the by-products formed by photocatalytic ozonation of DFC do not have toxicity against the tested strains, allowing the bacterial growth.

In order to improve the toxicity assay, different types of bioassays or biosensors could be implemented. For example, a test based on the inhibition of the bioluminescence of luminescent bacteria *Vibrio fischeri* or *Photobacterium phosphoreum* could be used. Other devices such as Microtox from Azur Environmental, LUMIStox from Beckman Instruments or Tox-Alert from Merck, which are based on the same principle, could also be used. This type of tests have some disadvantages, *V. fischeri* is a marine bacteria and in order to obtain reliable results a filtration will be required before the test and it works just in saline solution. The insolubility of some organic compounds can be enhanced because of the salinity. Biosensors, analytical devices combining a biological sensing element (enzyme, DNA or a microorganism) with a transducer, converting a biological signal into a measurable signal, could be applied. Biosensors gained interest because of

the possibility of mass production, fast response and great adaptability on-line monitoring. Different types can be chosen, for example, changes in bacterial UV absorption, inhibition of the conductivity of a polymer coated with an agarose layer containing immobilized *Saccharomyces cerevisiae* or the amperometrix system [29, 62].

For AMX and DFC, by the results in Figure 15 (c) and (d), a similar variation in the biomass yield was verified for the bacterial growth. For samples containing AMX it was concluded that the biomass yield is affected in the negative control as verified for the bacterial growth. This result confirms the antimicrobial power of AMX. For different times of reaction, it was once again confirmed the loss of toxicity after photocatalytic ozonation. For DFC the biomass yield for *E. coli* was not affected, while for *S. aureus* some effect was verified, in the negative control. These results show that the *S. aureus* strain used in this experiment was more sensitive to DFC than *E-coli*. Once again it is possible to conclude that different types of toxicity assays could be applied.

# 5 Conclusions and Future Perspectives

## 5.1 Conclusions

Complete degradation of AMX was achieved by single ozonation after 10 min of reaction while in photolytic and photocatalytic ozonation 5 min was enough. Relatively to AMX, < 5% was degraded by photolysis. On the other hand, DFC was completely degraded in all the reactions after 5 min.

In the different experiments a decrease in pH values due to the formation of by-products or $CO_2$ was often observed.

After 180 min of DFC photolysis no formation of both oxalic and oxamic acids was verified. Ozone by itself could not degrade the oxalic and oxamic acids. In photolytic and photocatalytic ozonation the oxalic and oxamic acids were degraded.

The values of total organic carbon demonstrated the occurrence of complete mineralization of AMX and DFC, after 30 and 120 min, respectively, for the photocatalytic ozonation.

For both AMX and DFC nitrogen presented in the parent molecules was converted to oxamic acid, nitrates, nitrites and ammonium. Similarly, it was observed the conversion of sulfur to sulfates for AMX and chlorine to chlorides for DFC.

After 15 minutes of photocatalytic ozonation, amoxicillin was no longer active against *Escherichia coli* and *Staphylococcus aureus.* The formation of toxic compounds as by-products resulting of the degradation of AMX and DFC did not occur.

It was concluded that the photolytic ozonation process is effective in disinfection of water, allowing the complete elimination of the bacteria *Escherichia coli*, after 180 min of treatment.

## 5.2 Future Perspectives

Some suggested future work:

- to perform photocatalytic ozonation experiments using the synthetic wastewater contaminated with both AMX and DFC pollutants and studying possible matrix effects.
- the concentrations of the two tested emerging pollutants, AMX and DFC, should be closer to those found in typical WWTPS; therefore, more sophisticated analytical techniques must be implemented;
- besides *E.coli*, different bacterial strains should be tested in the effectiveness of AOP on bacterial inactivation;
- different ozone concentrations and catalyst loads should be tested for optimization of the photocatalytic ozonation process;
- photolytic ozonation was effective in the inactivation of microorganism, thus the photocatalytic ozonation effectiveness is also expected but should be confirmed.
- in order to improve the toxicity assay, different types of bioassays or biosensors could be implemented.

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# Appendix A: Calibration curves of AMX, DFC, oxalic and oxamic acid, nitrates, nitrites, sulfates, chlorides, ammonium.

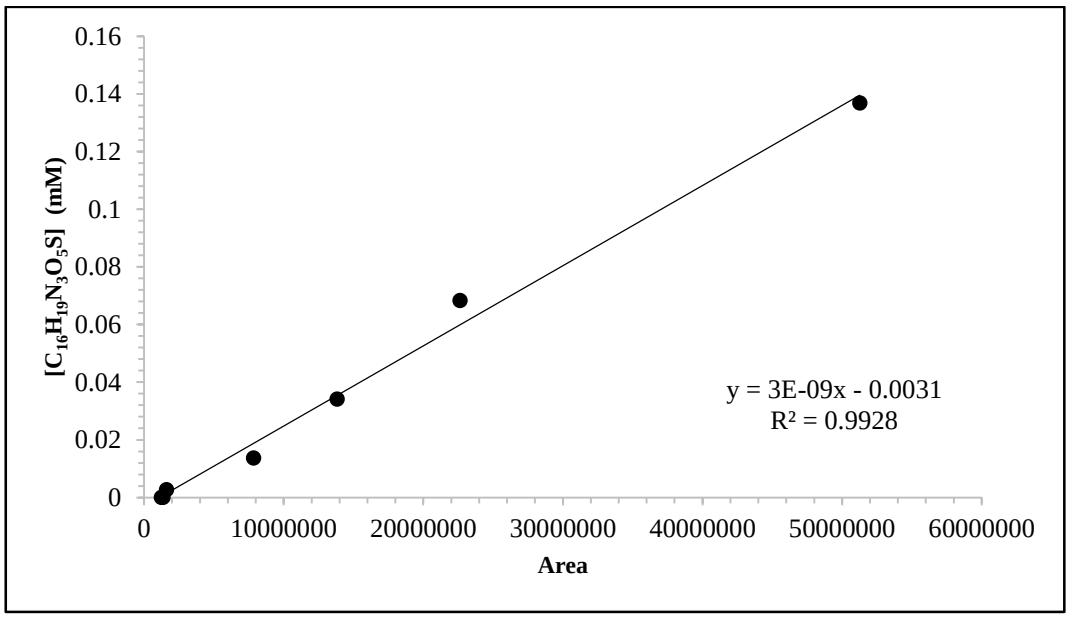


**Figure A1** – Calibration curve of AMX.

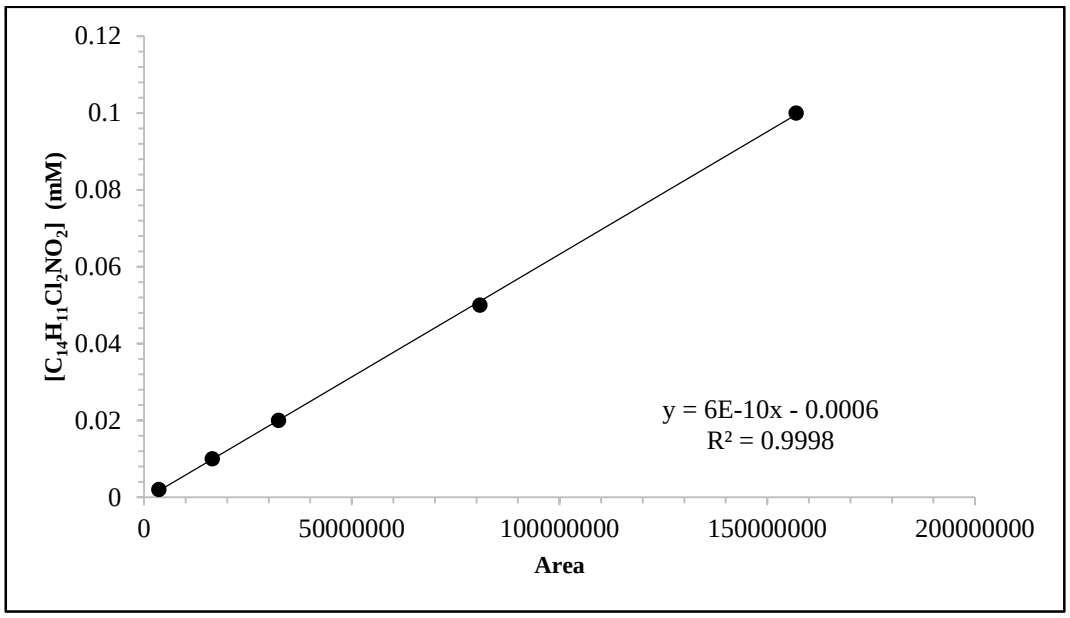


**Figure A2** – Calibration curve of DFC.

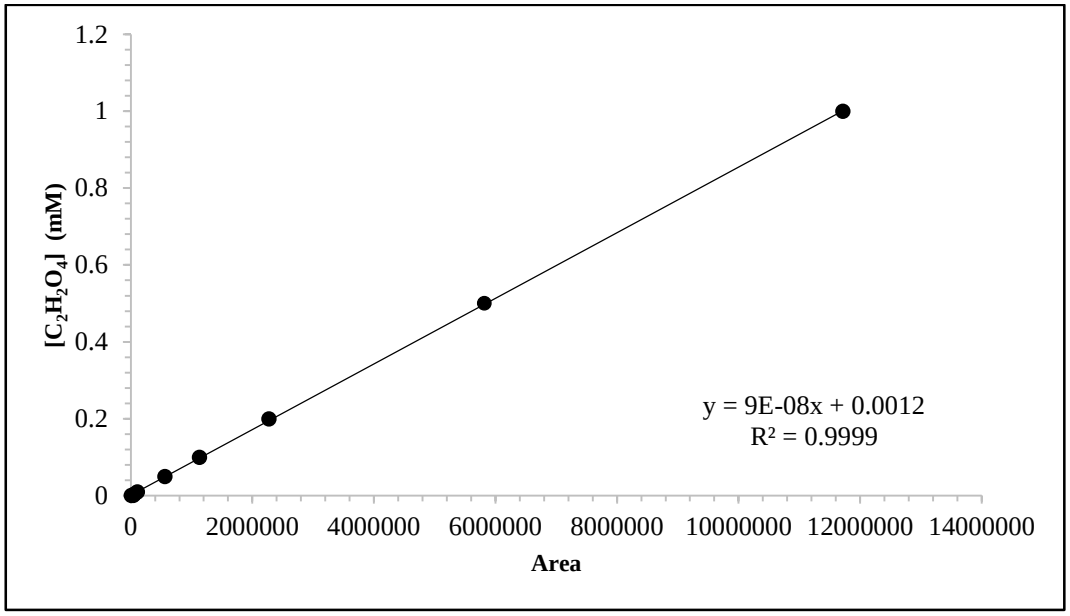


**Figure A3** – Calibration curve of oxalic acid.

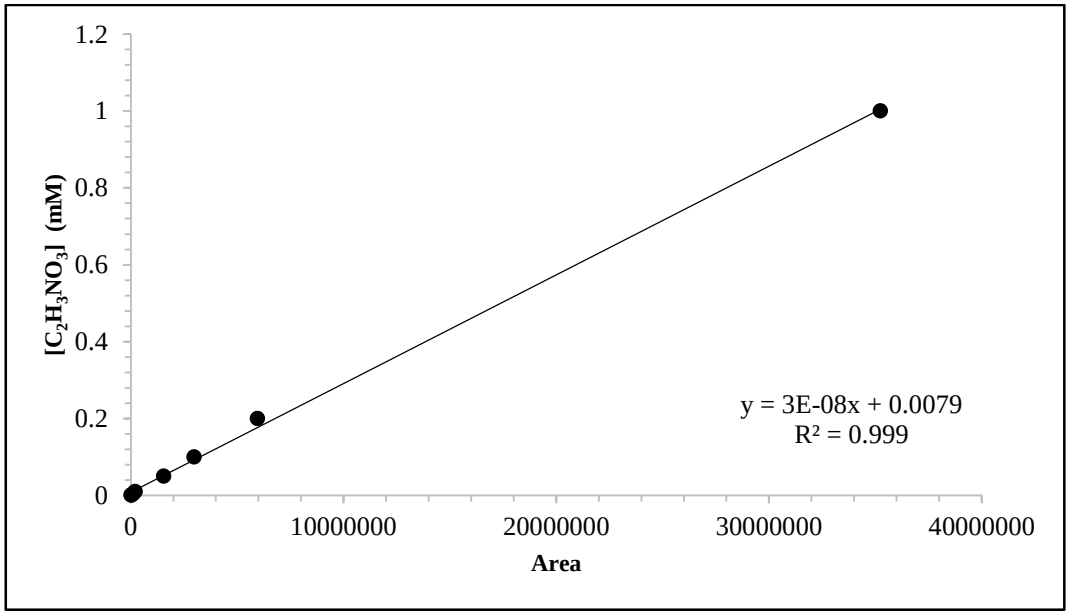


**Figure A4** – Calibration curve of oxamic acid.

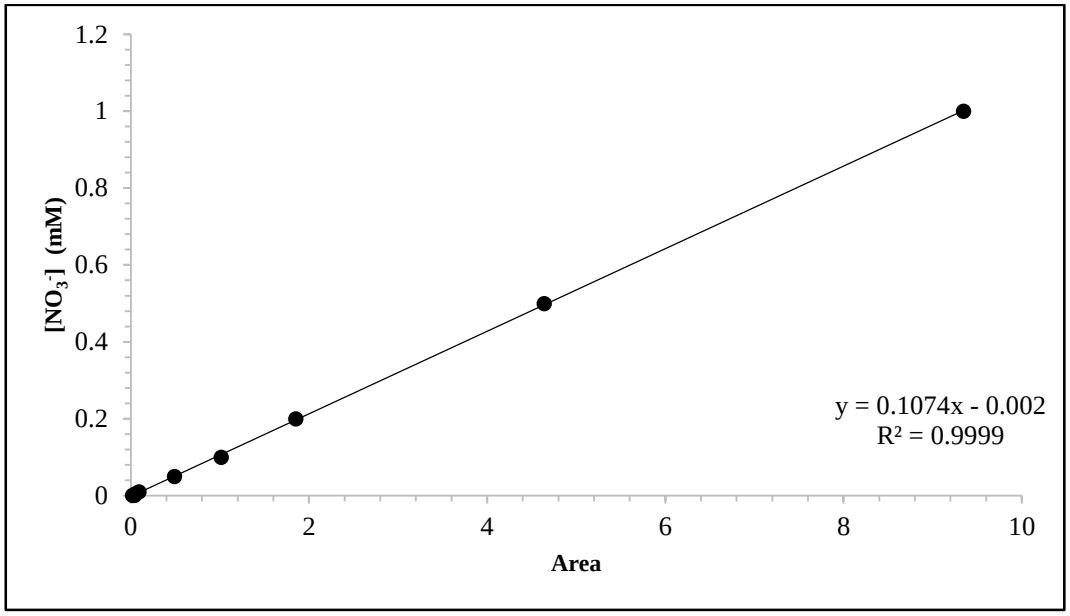


**Figure A5** – Calibration curve of nitrates

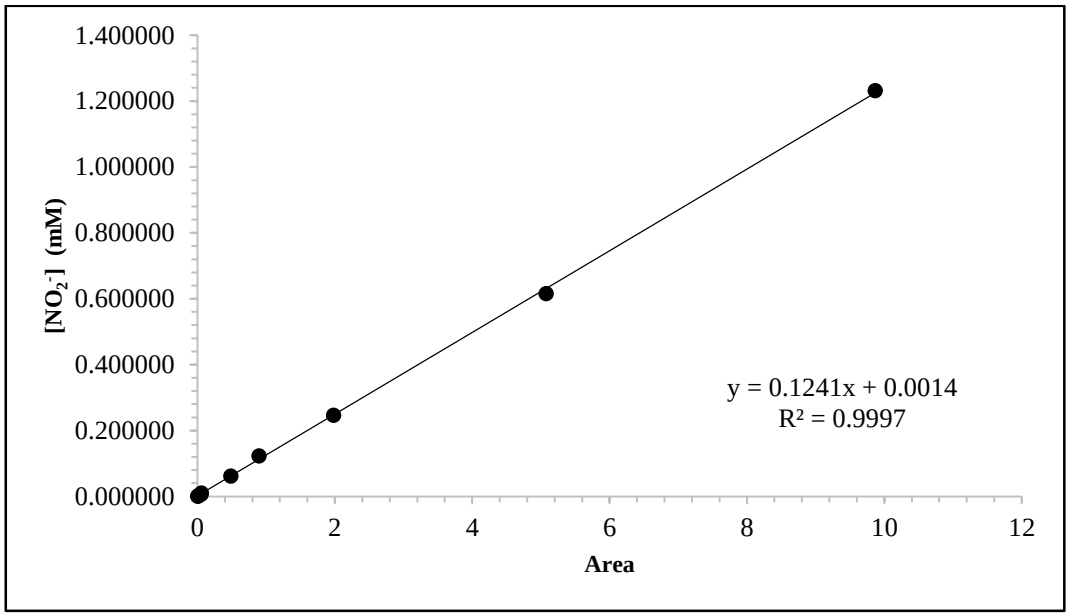


**Figure A6** – Calibration curve of nitrites

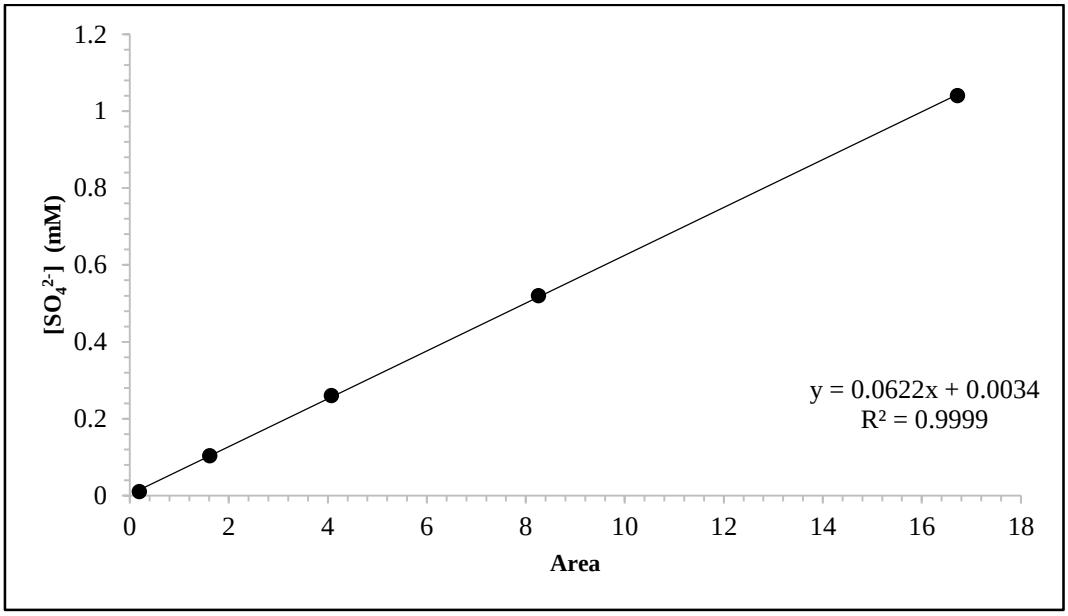


**Figure A7** – Calibration curve of sulfates

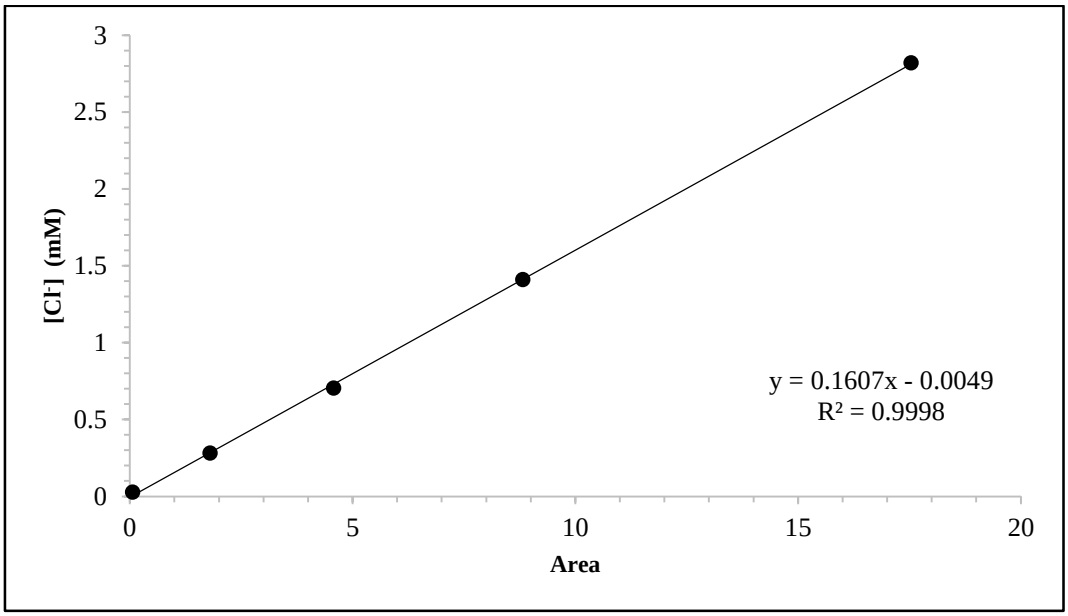


**Figure A8** – Calibration curve of chlorides

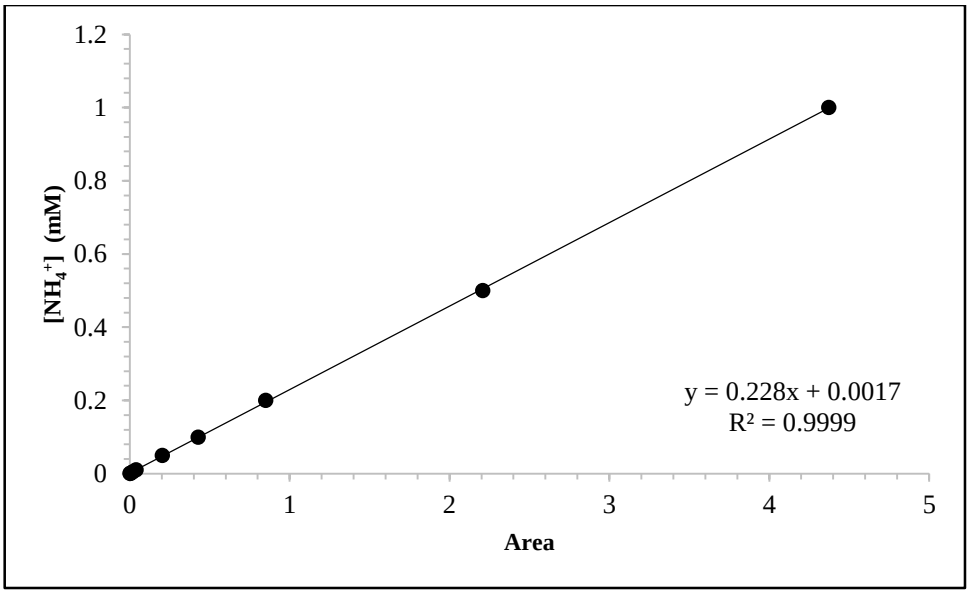


**Figure A9** – Calibration curve of ammonium.